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A CONSIDERATION OF CERTAIN REACTIONS OF STARCHES WITH SPECIAL REFERENCE TO ENZYME HYDROLYSIS[1]

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Introduction

It has been a widely prevalent belief that variabilities among starches are specific for their botanical origins. In accordance with this belief, laborious and painstaking attempts have been made to determine, in numerical units, values for boiling properties, gelatinization temperatures, digestibilities, and other constants of starches which would be specific of their botanical origins. But as a rule the values which have been found and proposed by different investigators have not been concordant or reproducible. Still the old notion of identity among starches of the same species has persisted, and not given way to the view that discordant values might be indicative of starch individualities which are independent of botanical classifications. However, in recent years, unsuspected individualities have been revealed among starches of the same botanical origin. Collatz (1922) and Rask and Alsberg (1924) have demonstrated differences among individual wheat starches, and Tadokaro and Sato (1923) have done likewise in the case of rice starches. Realization and recognition of the existence of such differences will probably cause investigators to abandon, more or less, past efforts for others of greater promise in attaining ultimate objectives in starch research. Among these are considerations of relations which may exist in the different properties of a starch or the influences which the different starch constants may have on one another. For such considerations most of the existing data are of little value, as they are on starches of unknown relations to one another and are therefore not comparable. The experimental work reported in this paper had for its original purpose the gath-

1 Abstracted from the thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Science in Hygiene, The Johns Hopkins University, Baltimore, Maryland.

ering of data which might serve for the considerations given. However, in its progress the work took such a turn as also to throw some new light on the nature of diastatic processes.

Materials

The material used in this investigation consisted of eleven starches prepared in this laboratory. Eight of these were wheat starches prepared from flours milled by the United States Department of Agriculture from unblended wheats grown at its own experimental stations. The remaining three were prepared from cornmeal, polished rice, and Irish potatoes, commercial products purchased in a local store. The wheats were grown and collected under the supervision of J. A. Clark, of the Office of Cereal Investigations, United States Department of Agriculture. These consisted of three samples of Kharkov, three of Minturki, one of Marquis, and one of Kota wheat. The three Kharkov samples and the three Minturki samples were progenies of the same seed of their respective varieties. Duplicate plots of Kharkov and Minturki were grown at North Platte, Nebraska; Moccasin, Montana; and Dickinson, North Dakota. The Marquis and Kota were grown at the last named station.

The meteorological conditions under which the wheats were matured are given in Table I. The data were supplied by the superintendents of the respective experimental stations.

TABLE I

METEOROLOGICAL DATA
(30-40 Days Before Ripening and Harvesting)

Experiment station	Mean air temperature	Precipitation	Sunshine
	Deg.	Inches	Per cent
North Platte, Neb.	67.85	2.84	88.0
Judith Basin, Moccasin, Mont.	60.75	1.89	63.3
Dickinson, No. Dak.	63.73	1.60	50.0

All the wheats were milled in an experimental mill of the United States Department of Agriculture, under the supervision of J. H. Shollenberger, of the Milling Division. W. K. Marshall of the same division conducted the baking tests by methods described in Department Bulletin No. 1187 (1923). The data of these milling and baking tests and of the results of chemical analysis as supplied by Mr. Marshall, are compiled in Table II. The chemical data were obtained by the official methods of the Association of Official Agricultural Chemists.

The meterological, baking, and chemical data contained in Tables I and II were accumulated and compiled in order to ascertain their relations to the chemical, physical, and biological constants which were to be determined in these starches.

TABLE II

RESULTS OF MILLING AND BAKING TESTS, AND CHEMICAL ANALYSIS OF THE MATERIALS FROM WHICH THE WHEAT STARCH SAMPLES WERE PREPARED

Sample No.	Class	Source	Milling yields			Water absorption of flour	Volume of loaf	Color of crumb	Texture of crumb	Basis, 13.5% moisture		
			Straight flour	Bran	Shorts					Crude protein in wheat (Nx5.7)	Ash content in flour	Moisture in flour
			%	%	%	%	cc.	Score	Score	%	%	%
11717	Dark hard Winter	Neb.	75.4	12.3	12.8	61.2	1770	85.5	86.3	13.0	0.40	13.2
11719	" " "	"	73.9	18.2	8.2	53.5	2080	87.0	90.5	13.2	0.38	13.3
11800	Hard Spring	No. Dak.	71.4	14.3	17.5	59.7	2080	92.0	89.9	11.1	0.45	13 4
11801	(smutty)	" "	78.7	15.3	9.7	64.7	2250	91.2	92.4	11.6	0.42	13.4
11901	Dark hard Winter	Mont.	80.2	13.5	9.0	62.6	1980	88.9	88.9	12.8	0.45	14.1
11904	" " "	"	79.9	14.3	7.8	57.6	2170	88.9	90.5	13.4	0.42	13.4
11948	" " "	No. Dak.	73.2	15.5	14.2	67.7	2170	87.9	89.5	11.9	0.38	13.7
11954	" " "	" "	76.3	15.9	9.5	60.0	2420	87.3	89.5	13.9	0.40	13.7

Preparation and Purification of Starches

All wheat starches were prepared, purified, and desiccated according to the technic described by Rask and Alsberg (1924). For other starches the same technic was employed, with such special modifications as each required.

In preparing the potato starch, the potatoes were washed, peeled, cut into small pieces, and ground in an ordinary hand grinder with a small but constant stream of distilled water. The starch was removed from the resulting pulp by straining through cheese-cloth. From this point its purification was identical with that of wheat starches.

In preparing the rice starch, the white, polished grains were given a preliminary soaking for one hour in distilled water, after which they were ground in the hand grinder with a small stream of distilled water. A thick and milky suspension resulted, from which the starch was separated by straining through cheese-cloth, and purification was continued by means of the centrifuge, as with the wheat starches.

Maize starch was prepared from cornmeal in a manner identical with the preparation of potato and rice starches, except that the cornmeal was triturated with water in a mortar instead of ground in the grinder.

The purity of starches prepared in this manner may be judged by their nitrogen and moisture contents, which are given in Table III. It will be noted that the nitrogen contents vary from 0.032 to 0.046 per cent in wheat starches. The nitrogen content of corn starch was the highest, being 0.265 per cent.

TABLE III

MOISTURE AND NITROGEN CONTENT OF STARCH SAMPLES

Sample No.	Moisture			Nitrogen		
	1	2	Av.	1	2	Av.
	%	%	%	%	%	%
11717	4.25	4.21	4.23	0.0364	0.0376	0.0370
11719	4.25	4.23	4.24	.0224	.0168	.0196
11800	5.81	5.81	5.81	.0260	.0440	.0350
11804	4.40	4.54	4.47	.0300	.0500	.0400
11954	4.34	4.70	4.52	.0440	.0480	.0460
11948	4.18	4.49	4.38	.0300	.0300	.0300
11901	5.18	5.26	5.22	.0360	.0440	.0400
11904	4.64	5.14	4.89	.0400	.0400	.0400
Maize	8.31	8.56	8.43	.2600	.2700	.2650
Potato	5.81	5.82	5.815	.0500	.0600	.0550
Rice	5.09	5.31	5.20	0.1460	0.1500	0.1480

Acids and alkalis were not used in the purification of these starches because of the possibility that such treatment might denature the starches or alter their properties. This seemed particularly true of alkalis, because starches have an acid reaction and are therefore capable of combining with alkalis. Strange to say, this possibility has been overlooked by many other investigators who have used alkalis in the purification of starches.

Experimental

The following constants and properties of these starch samples were determined.

1. Viscosities of their pastes at 90° and at concentrations ranging from 0.6% to 5.5%. From these results viscosity equations were computed for each starch.
2. Their relative resistances in the raw or ungelatinized state to amyloytic action.
   (a) When excess or surplus quantities of starch were exposed to limited quantities of malt diastase. The extent of action under this condition was measured by the amounts of maltose produced.
   (b) When exposed in limited quantities to excessive quantities of: (1) malt diastase; (2) a commercial preparation of pancreatic amylase. The extent of action under this condition was measured by the amounts of starch left unattacked or "undissolved."
3. The relative extent to which they were acted on or attacked in the cooked or gelatinized state by the same commercial preparation of pancreatic amylase. In this case the extent of action was measured by decrease in the viscosity of the paste.

Viscosity of the pastes of these starches were determined in centipoise units according to the technic of Rask and Alsberg (1924). As this technic requires a Stormer viscosimeter, its calibration was a preliminary step. Water and sucrose solutions of 20, 40, and 60% concentrations were used for standards. Their absolute viscosities at convenient working temperatures have been determined by Bingham and Jackson (1917), whose values were used in this calibration. The sucrose used was obtained from Merck and Company, marked "C.P. blue label." The method of calibration differed from that of Rask, and Alsberg in that three weights were used to propel the moving parts of the viscosimeter

namely, 100, 150, and 200 gm. As the calibrating technic was identical with that of subsequent viscosity determinations of starch pastes, it will be worth while to describe it in some detail at this point.

The viscosimeter cup was filled to a certain mark with the viscous substance, so that the latter covered the rotating cylinder to a depth of 0.6 or 0.7 cm. when the viscosimeter cup was raised into position for operation. After this was done, the thermometer was placed in its position within the cup and the cup covered with

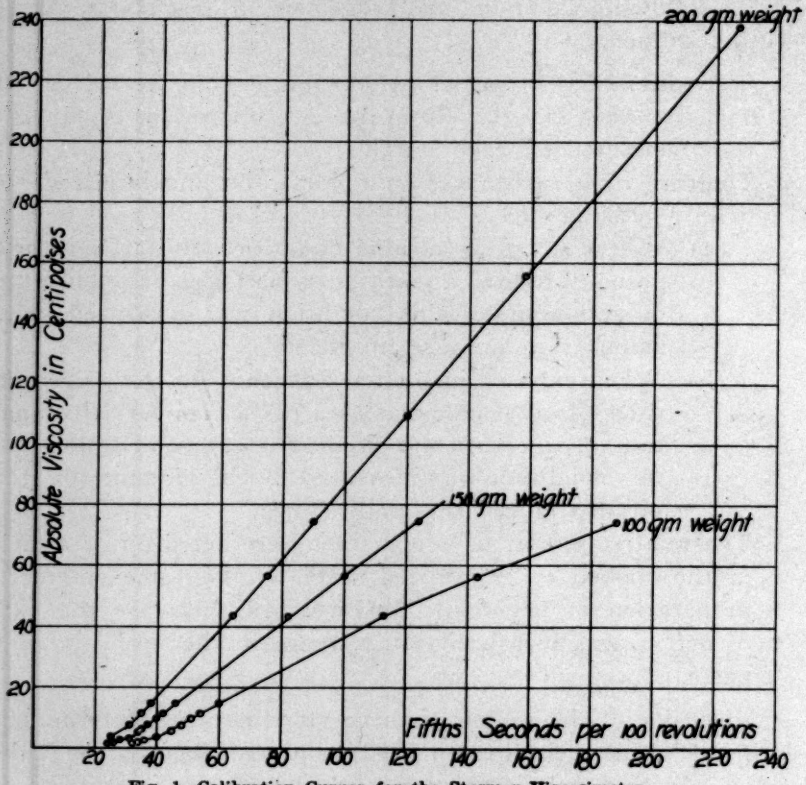

Fig. 1. Calibration Curves for the Stormer Viscosimeter

a sheet metal cover to reduce evaporation. The pointer of the revolution counter was next brought to the mark indicating 8 or 10 revolutions back of the zero point, in order that the rotating cylinder rotated that many times before starting the timer. Then the weight was raised into position by winding on the drum the string attached to it. In the meantime, the temperature of the bath was adjusted by either a gas burner underneath or ice within,

depending upon the temperature desired. This crude means of control supplemented by patience and a little skill, served to maintain the bath within one-half degree of the required temperature. When this was attained everything was ready for a determination, which consisted essentially of noting the time required for 100 revolutions of the rotating cylinder. The weight was released, and as the pointer of the revolution counter passed the zero mark, the stop watch, or timer, was started. As the pointer passed the zero mark again, thereby indicating 100 revolutions, the watch was stopped. It is important to note that the rotating cylinder must be allowed 8 or 10 revolutions for overcoming inertia, so that the measured speed was due entirely to the viscosity of the substance. Calibration data obtained by this technic are contained in Table IV. From these data the calibration curves given on Plate 1 were drawn.

TABLE IV

STANDARDIZATION OF STORMER VISCOSIMETER

	Temperature °C.	Absolute viscosity in centipoise	No. of 1-5 seconds per 100 revolutions with 100-gm. weight	with 150-gm. weight	with 200-gm. weight
Sucrose 20%	0	3.80	41	33	25
	5	3.15	40	31	25
	10	2.65	37	29	24
	15	2.67	36	28	23
	20	1.96	34	26	..
	25	1.70	32	24	..
Sucrose 40%	0	14.77	60	46	38
	5	11.56	54	42	35
	10	9.79	51	40	32
	15	7.468	48	37	31
	20	6.200	46	35	30
	25	5.187	45	34	30
Sucrose 60%	0	238.0	..	..	227
	5	156.0	..	..	158
	10	109.8	..	..	120
	15	74.6	188	124	90
	20	56.5	143	100	75
	25	43.86	113	82	64

Interpolated from the values of Bingham and Jackson (1917).

Attention is called to the shape of these curves. It will be noticed that the curves for the 150- and 200-gm. weights are straight lines, showing that in the case of these weights and within the limits in which they were used, viscosity is a linear function of time required for the rotating cylinder to make 100 revolutions. However, the curve for the 100-gm. weight has the shape of an

elongated S. The deviation from the straight line at the lower end is probably caused by the high speed and consequent momenta of the rotating cylinder and the falling weights as a result of a low viscosity. The upper bend is probably due to variabilites or irregularities in the friction of the viscosimeter mechanism, which becomes significant as the speed of the cylinder and weights falls below a certain point. Subsequent experiments have shown the advisability of confining viscosity measurements to ranges within which the calibrating curves are straight lines. Outside of these ranges, especially in the region of the upper bend of the 100-gm. weight curve, it was somewhat difficult to obtain duplicates which checked. For this reason it is advisable to use larger weights than 100 gm. for determining viscosities greater than 100 centipoises.

TABLE V

VISCOSITIES OF THE STARCHES

Sample No.	Concentration	Time required for 100 revolutions		Viscosity
	%	Seconds/5	Seconds/5	Centipoise
11717	3.35	47	48	7.4
	4.31	70	71	22.0
	5.27	113*	113*	67.2
11719	3.35	45	45	5.0
	4.31	62	61	16.5
	5.27	98*	97*	55.5
11800	3.3	48	48	7.8
	4.24	74	74	24.0
	5.18	176	175	70.2
11804	3.34	45	44	5.0
	4.3	59	60	14.77
	5.26	83	83*	44.0
11954	3.34	44	43	4.5
	4.3	60	61	14.77
	5.26	115	114	45.0
11948	3.35	43	41	4.0
	4.31	60	58	14.5
	5.26	109	112	42.3
11901	3.32	46	46	6.0
	4.27	65	64	18.0
	5.22	139	139	54.9
11904	3.34	48	49	7.8
	4.28	74	74	24.0
	5.24	166	166	66.0
Maize	3.21	61	61	15.8
	4.13	70	71	51.5
	5.04	160**	159**	159.0
Potato	.94	113	113	43.5
	1.41	109**	113**	102.6
	1.77	197**	196**	201.5
Rice	3.32	86	85	32.0
	4.27	118*	120*	71.0
	5.22	174**	176**	177.4

*Obtained by 150-gm. weight.
**Obtained by 200-gm weight· all others by 100-gm. weight.

Comparative viscosities of the starches.—Viscosities of pastes of each starch at 90° C. and at three different concentrations, ranging from 1.0 to 5.5 per cent, were then determined by the calibrated Stormer. As the details of the technic have been described by Rask and Alsberg (1924), they will not be repeated here.

Results.—The resulting data are compiled in Table V. In this table the duplicates represent average viscosities in stop watch readings of fifth-seconds of two separate pastes or preparations. The unmarked readings were obtained by the 100-gm. weight. The readings marked by a single asterisk were obtained by the 150-gm. weight; and those marked by double asterisks, by the 200-gm. weight. The averages of the duplicates were used for conversion into centipoise units. This was done by means of the calibration curves on Plate 1.

Attention may be directed to the relatively high viscosities displayed by the pastes of the potato starch. In Table VI it will be noticed that the computed viscosity of a 4% paste of this starch is 12485 centipoises as compared with 65.13 centipoises, the viscosity of the next highest paste of this concentration. However these high viscosities were only temporary. They fell to extremely low and irregular values within five minutes. In this respect potato starch pastes differed from the pastes of all other starches whose viscosities, as recorded in Table V, were permanent. Microscopic examinations of the pastes of the several starches after staining with congo red showed that the paste of the potato starch was composed of ruptured grains, whereas the pastes of all the other starches were composed of unruptured grains. The temporarily high but subsequently low viscosities of potato starch pastes indicate that potato starch grains in the process of gelatinization swell to an exceptionally high degree and then rupture. Further reference to this exceptional behavior of the potato starch will be made later.

Rask and Alsberg have pointed out that relationships between viscosities of starch pastes and their concentrations can be represented by the equation:

$$\log y = mx + \log b$$

In this equation $y =$ viscosity, $x =$ concentration of starch, and m and b are constants.

The numerical values of these constants for each starch may be computed from the data given in Table V by means of the following equations:

$$N(\log b) + (\Sigma x)\ m = \Sigma \log y$$
$$(\Sigma x)\ (\log b) + (\Sigma x^2)m = (\Sigma x)\ \log y$$

N = number of observations.

These constants so computed are given in Table VI. They were determined in order to ascertain their relationships to other values determined in the course of this investigation.

TABLE VI

COMPUTED VALUES OF M AND B AND VISCOSITIES AT 4 PER CENT

Sample No.	m	log b	b	Viscosities at 4%
11717	0.4884	−0.7575	0.1748	15.7
11719	.5425	−1.1192	.0769	11.24
11800	.4490	−.5290	.2958	18.50
11804	.4921	−.9454	.1134	10.55
11954	.5210	−1.0817	.08285	10.05
11948	.5363	−1.1784	.06631	9.26
11901	.3260	−.1344	.7338	14.78
11904	.4849	−.7123	.1940	16.88
Maize	.5465	−.5512	.2811	43.13
Potato	.8024	.8868	7.7055	12485.00
Rice	0.1655	1.1518	14.185	65.13

Relative Resistances of Starches in Raw or Ungelatinized Condition to Malt Diastase when Exposed in Surplus Quantities to Limited Quantities of the Enzyme

Resistances under these conditions were measured by the amounts of maltose produced as a result of diastatic activity. This procedure requires an efficient diastatic inhibitory agent. Among the possible diastatic inhibitory agents the following were considered:

Phenol, liquefied, of 85% concentration
Chloroform
Tungstic acid (Na_2WO_4 and normal H_2SO_4).

Tungstic acid was tried because of the success with which Rumsey (1922) had used it for precipitating wheat flour proteins and inhibiting diastatic action. Phenol and chloroform were tried because they are frequently used and recommended for inhibiting enzyme actions. The following experiments show their relative efficiencies.

Relative inhibitory powers of different reagents.—Experiment No. 1. Three grams of starch were shaken in a 100-cc. volumetric flask, with 25 cc. of distilled water, and 20 cc. of freshly prepared malt extract, both previously brought to 40° C. The flask was then allowed to remain for one hour in a thermostat maintained at 40° C. During this time it was shaken once every 15 minutes. At the end of the hour six drops of thymol blue (0.4%), according to Clark (1922), 1 cc. of 15% of Na_2WO_4, and 1.5 cc. $\frac{N}{1}$ H_2SO_4 were added in the order named. Addition of $\frac{N}{1}$ H_2SO_4 was continued drop-wise until ten drops had been added in excess of that required to change the color of the indicator. This is the acidity for inhibiting diastatic action, as specified by Rumsey (1922). The mixture was then brought to the mark with distilled water and shaken thoroly to produce uniform solution and also an immediate inhibition of diastatic action. The mixture was next centrifuged to separate the unhydrolyzed starch. Fifty cc. of clear supernatant solution was then pipetted into a 150-cc. Erlenmeyer flask, to which 5 cc. HCl (sp. gr. 1.125) was added. This mixture was digested for two and a half hours on a steam bath, using a reflux condenser to prevent concentration. This digestion converts starch hydrolysis products into dextrose. At the end of the two and a half hours the solution was neutralized with 10% NaOH and made up to 100 cc. in a volumetric flask. Dextrose was determined in a 50-cc. aliquot of this solution by the Munson-Walker method given in the A. O. A. C. manual (1925).

Experiment No. 2. This experiment was identical with No. 1, except that 5.0 cc. of liquefied phenol (85%) was used as an inhibitory agent in place of the Na_2WO_4 and H_2SO_4 solutions. The resulting solution was somewhat cloudy and smelled of phenol.

Experiment No. 3. This experiment was the same as No. 1, except that 1 cc. of chloroform was used as an inhibitory agent in place of the Na_2WO_4 and H_2SO_4 solutions. The resulting mixture was fairly clear and emitted a distinct odor of chloroform.

Experiment No. 4. This was the blank, or control. It was identical with Experiment No. 1, except that no starch was used. The resulting solution was very clear, like that of Experiment No. 1, indicating excellent clarification.

All these experiments were conducted in duplicate. The results are given in Table VII.

TABLE VII

RELATIVE EFFICIENCIES OF DIFFERENT SUBSTANCES FOR INHIBITING DIASTATIC ACTION OF MALT EXTRACT

Exp. No.	Starch	Final vol.	Inhibitory agents		Wt. Cu_2O per 50 cc. of Sol. (0.75 gm. starch)	Corresponding dextrose	Clarification
	gm.	cc.	Kinds	cc.	gm	mg.	
1	3	100	Na_2WO_4, 15%	1.0			
			$\frac{N}{1}$ H_2SO_4	1.5			
			$\frac{N}{1}$ H_2SO_4 excess	0.6	0.0661	28.3	Excellent
			by thymol blue	0.3			
	3	100	Na_2WO_4, 15%	1.0			
			$\frac{N}{1}$ H_2SO_4	1.5			
			$\frac{N}{1}$ H_2SO_4 excess	0.6	0.0663	28.3	Excellent
			by thymol blue	0.3			
2	3	100	Liq. phenol	5.0	0.0692	29.6	Cloudy
	3	100	Liq. phenol	5.0	0.0761	32.7	Cloudy
3	3	100	Chloroform	1.0	0.1113	48.2	Fair
	3	100	Chloroform	1.0	0.1135	49.1	Fair
4	0.0	100	Na_2WO_4, 15%	1.0			
			$\frac{N}{1}$ H_2SO_4	1.5			
			$\frac{N}{1}$ H_2SO_4 excess	0.6	0.656	28.05	Excellent
			by thymol blue	0.3			
	0.0	100	Na_2WO_4, 15%	1.0			
			$\frac{N}{1}$ H_2SO_4	1.5			
			$\frac{N}{1}$ H_2SO_4	0.6	0.0660	28.3	Excellent
			by thymol blue	0.3			

Discussion.—In Table VII it will be noticed that the same weights of Cu_2O were obtained in Experiments Nos. 1 and 4. This may be regarded as proof that diastatic action was completely inhibited by tungstic acid under the condition prescribed in these experiments. The higher results produced in Experiments Nos. 2 and 3 show that phenol and chloroform are not satisfactory diastatic inhibitory reagents. Attention may also be called to the fact that tungstic acid produced a better clarification than either phenol or chloroform. Accordingly, tungstic acid was selected as the diastatic inhibitory agent in determining from the amounts of reducing sugars produced the relative resistances of starches in raw or ungelatinized condition to amylotytic action, when exposed in surplus quantities to diastase.

However, before the final details of the technic had been arranged, it was considered worth while to test the efficiency of the acidity of the above inhibited mixture for hydrolyzing maltose into dextrose. If this acidity should be found sufficient for hydrolyzing maltose into dextrose under the condition that HCl of sp. gr. 1.125 is used for this purpose, the addition of the latter might be eliminated and the technic simplified accordingly. The test was carried out as follows:

Two hundred and fifty cc. of distilled water previously heated to 40° C. was added to approximately 30 grams of starch contained in a 500-cc. Erlenmeyer flask. The flask was shaken in order to produce a uniform suspension of its contents, after which 100 cc. of freshly prepared malt extract was added and allowed to act for about an hour at 40° C. At the end of this time the mixture was centrifuged in order to obtain a clear solution of maltose produced under the condition of subsequent experiments. A 50-cc. portion of this maltose solution was pipetted into each of five Erlenmeyer flasks. Five cc. of HCl (sp. gr. 1.125) was added to each of two of these flasks. The contents of a third flask were used for a preliminary test, the purpose of which was to ascertain the amount of $\frac{N}{1}$ H_2SO_4 required to produce a change in the color of thymol blue, plus 5 drops in excess after 0.5 cc. of 15% Na_2WO_4 had been added. To each of the other two flasks of maltose solution 0.5 cc. of 15% Na_2WO_4 was added, followed by a volume of $\frac{N}{1}$ H_2SO_4 equal to that required to produce the desired acidity as indicated in the above preliminary experiment with the contents of the third flask. The contents of these four flasks were then digested on a steam bath for two and a half hours, using a reflux condenser to prevent losses by evaporation. At the end of this digestion period the

contents were neutralized with 10% NaOH and diluted to 100 cc. in a volumetric flask. Reducing sugars were determined in 50-cc. aliquots according to the Munson-Walker method. The averages of the resulting data are compiled in Table VIII.

TABLE VIII

RELATIVE HYDROLYZING ACTIONS OF HCl (SP. GR. 1.125) AND H_2WO_4 ON 50CC. PORTIONS OF A MALTOSE SOLUTION

Portion	Na_2WO_4, 15%	Acids used	Wt. of 50-cc. solution	Dextrose
		cc.	gm.	mg.
1			0.2121	94.6
2	0.0	5 cc. HCl	0.2137	95.1
4		1.4 cc. $\frac{N}{1}$ H_2SO_4	0.1083	46.9
5	0.5	+5 drops in excess thymol blue.	0.1078	46.4

Discussion.—From Table VIII it will be noticed that the solutions digested with HCl yielded almost twice as much dextrose as those digested with Na_2WO_4 and H_2SO_4. As the reducing power of maltose is approximately twice that of dextrose, it may be concluded from these data that H_2SO_4 in the above concentrations and under the conditions of the experiment, has practically no hydrolyzing action on maltose. Accordingly, 5 cc. of HCl must be added for this purpose.

Determination of Relative Resistances of Raw Starches to the Action of Malt Diastase When Exposed to Excessive Quantities of Enzyme

Guided by the results obtained in the preceding preliminary tests of inhibitory and hydrolyzing agents, a method was evolved for estimating relative resistances of raw starches to malt diastase, as measured by the amount of reducing sugars produced. The following reagents are required:

Thymol blue, 0.4%
Sodium tungstate, 15% of Na_2WO_4
Sulphuric acid, C.P. — normal solution
Malt extract.

The malt extract was prepared by the A. O. A. C. method. Twenty grams of freshly pulverized malted barley was digested with 400 cc. of distilled water in an Erlenmeyer flask for two hours with occasional shaking. At the end of two hours the mixture was

filtered through a single filter paper. A dextrose determination of a definite quantity of the extract for correction was not determined.

The details of the technic are as follows: Three grams of each starch are suspended in 25 cc. of distilled water in a 100-cc. volumetric flask. The contents of the flask are brought to 40° C. by immersion in the thermostat maintained at this temperature, after which 20 cc. of malt extract previously brought to 40° C. is added. At the end of one hour, diastatic action is halted by the addition of 0.5 cc. of a 15% solution of sodium tungstate and normal sulphuric acid, 10 drops in excess of that required to change the color of thymol blue, according to the technic already described. The contents of the flask are next made up to volume, mixed thoroly, and contrifuged. Fifty cc. of the clear supernatant solution is transferred to a 150-cc. Erlenmeyer flask and digested with 5 cc. of HCl (sp. gr. 1.125) for two and a half hours on a steam bath, using a reflux condenser to prevent evaporation. This is the procedure of the A. O. A. C. for hydrolyzing maltose into dextrose. The contents of the flask are next carefully neutralized with 1% NaOH and made up to 100 cc. in a volumetric flask. Dextrose is then determined on the 50-cc. aliquot according to the Munson-Walker method.

This technic was applied in duplicate to all the starches simultaneously, using the same portion (20 cc.) of the same solution of malt extract in order that the results obtained on the starches might be strictly comparable. A five-minute interval was allowed between the additions of malt extract to each flask, and the same length of time between the additions of inhibitory agents, so that every flask was exposed to the action of malt extract for exactly one hour. The results are recorded in Table IX in terms of the weight of Cu_2O produced per 50-cc. aliquot. They represent, therefore, one-fourth of the soluble carbohydrates produced from the original 3 gm. of starch. These results have also been converted into their equivalent amount of dextrose. Averages of the duplicates are compiled in Table IX.

Discussion of the data contained in this table will be deferred to a later section, in which it will be considered in its relation to other data which were obtained in the course of this investigation.

TABLE IX

ACTIONS OF LIMITED AMOUNTS OF MALT DIASTASE ON EXCESSIVE AMOUNTS OF RAW STARCHES (ONE HOUR AT 40° C.)*

Purified starches	Class	Sample No.	Source	Per 50 cc. of digestion solution	Computed as dextrose from 0.75 gm. of starch
				Cu_2O gm.	mg.
Kharkov	H. R. W.	Wheat 11717	North Platte, Neb.	0.0710	30.50
Minturki	H. R. W.	Wheat 11719	North Platte, Neb.	.0315	13.30
Marquis	H. R. S.	Wheat 11800	Dickinson, No. Dak.	.0698	29.92
Kota	H. R. S.	Wheat 11804	Dickinson, No. Dak.	.0864	37.26
Kharkov	H. R. W.	Wheat 11901	Dickinson, No. Dak.	.0601	25.64
Minturki	H. R. W.	Wheat 11904	Dickinson, No. Dak.	.0354	14.96
Kharkov	H. R. W.	Wheat 11948	Moccasin, Mont.	.0677	28.98
Minturki	H. R. W.	Wheat 11954	Moccasin, Mont.	.0472	20.08
Maize, commercial				.1425	62.40
Potato, Irish, commercial				.0367	15.48
Rice, polished, commercial				0.1089	47.26

*The results are corrected by the blank tests.

Resistance of Raw Starches to Amylolytic Actions when Exposed in Excessive Quantities to Limited Quantities of Enzymes

In the preceding technic it will be noted that a large excess of starch was used. In the case of the most easily hydrolyzed starch (corn), only 250 of the original 3000 mg., or less than 9%, was hydrolyzed into water-soluble products. Under such conditions the enzyme has an opportunity to select the least resistant portion of the starch grains for action, hence the results obtained may indicate relative resistances of only those portions of the starch grains which have been selected by the enzyme rather than the relative resistances of the entire grain. In order to ascertain the latter, it will be necessary so to arrange conditions that an enzyme supply will be available for the entire grain, or an excess of enzyme and a limited quantity of starch present, so that this large quantity of enzyme will be compelled to attack the entire grain in place of selected or choice areas. Under such conditions the entire starch grain will be exposed or subjected to enzyme action and the amount of each starch which remains unaffected can be a measure of the relative resistance of the entire grains.

In order to ascertain satisfactory conditions for such procedure, the following preliminary experiments were performed:

Five-hundred-milligram portions of the same starch were transferred quantitatively to each of six 150-cc. Erlenmeyer flasks. These were subjected to the action of the same malt extract at 40° C., and under such varying conditions as are indicated in Table X. At the end of the different time periods shown in the table, the residual starch was filtered off quantitatively on a pre-

viously prepared and weighed Gooch crucible, washed with distilled water, then with alcohol, and finally with ether, dried at 100° C., cooled, and weighed. These weights and the weights of hydrolyzed starch obtained by subtracting from the original 500 mg. the unattacked residues, are recorded in Table X.

TABLE X

ACTION OF MALT EXTRACT ON LIMITED QUANTITIES OF RAW STARCH

Exp. No.	Weight of starch used	Amount of malt extract used	Time of digestion at 40° C.	Weight of residual starch	Weight of starch hydrolyzed into maltose
	mg.	cc.	hr.	mg.	mg.
1	500	50	1	456.7	43.3
2	500	20	1	453.2	46.8
3	500	50	2	447.9	52.1
4	500	20	2	441.1	58.9
5	500	50	3	436.0	64.0
6	500	20	3	436.2	63.8

The data in Table X show that in digestions ranging between one and three hours, 20 cc. of malt extract will hydrolyze practically as much of 500 mg. of raw starch as will 50 cc. of the same malt extract. Accordingly, it cannot be said that the extent of diastatic action is always proportional to the amount of malt extract present. In this case, anything in excess of 20 cc. appears to be a surplus. However, the data of Table X show that time is a factor. There is a progressive increase in the amount of starch hydrolyzed as the time during which it is exposed to enzyme action is increased from one to three hours.

From a consideration of the results of these preliminary experiments (Table X), the following technic was selected for ascertaining the relative resistance of raw starches to diastatic action when exposed in a limited quantity to a surplus of diastatic enzymes.

Duplicate 500-mg. portions of all starches were transferred quantitatively into 150-cc. Erlenmeyer flasks. At intervals of five minutes, 25-cc. portions of the same malt extract, freshly prepared and previously brought to 40° C., were added to each flask in a recorded order. From each flask the starch residue which remained at the end of three hours from the time the malt extract had been added was transferred quantitatively to previously prepared and weighed Gooch crucibles. In this procedure the flasks were taken in the order in which the malt extract had been added. The filtration of each flask was started five minutes after that of the preceding flask. In this manner each 500-mg. portion was ex-

posed to enzyme action for exactly the same length of time, that is, three hours. The residues on the Gooch crucibles were washed with distilled water, alcohol, and finally with ether, dried at 100° C., cooled in a desiccator, and weighed. The averages of the results are recorded in Table XI.

TABLE XI

RESIDUES OF RAW STARCHES AS A RESULT OF THE ACTIONS OF MALT DIASTASE AND PANASE

Sample No.	Malt diastase			Panase		
	Wt. of starch used	Wt. of residual starch	Wt. of starch hydrolyzed	Wt. of starch used	Wt. of residual starch	Wt. of starch hydrolyzed
	mg.	mg.	mg.	mg.	mg.	mg.
Wheat 11717	500	436.9	63.1	500	437.3	62.7
Wheat 11719	500	460.0	40.0	500	455.05	44.95
Wheat 11800	500	440.2	59.8	500	449.15	50.85
Wheat 11804	500	439.5	60.5	500	446.3	53.70
Wheat 11954	500	458.5	41.5	500	450.5	49.50
Wheat 11948	500	443.3	56.7	500	440.15	59.85
Wheat 11901	500	448.9	51.1	500	444.1	55.9
Wheat 11904	500	459.3	40.7	500	452.45	47.55
Maize, commercial	500	406.8	93.2	500	378.95	121.05
Potato, white, commercial	500	468.0	32.0	500	477.4	22.6
Rice, commercial	500	402.8	97.2	500	358.9	141.1

In considering the relative resistance of raw starches to diastatic action, it seemed advisable to include some other enzyme than malt diastase. As such, pancreatic amylase seemed especially worthy of consideration, because the resistance of a starch to this particular enzyme would probably be of the same order as its resistance to digestion in the digestive tract. Accordingly, an examination was made of the relative resistances of these starches to pancreatic amylase. A commercial preparation of pancreatic amylase labeled "Panase," and supplied by Frederick Stearns and Company, was used for this purpose. Relative actions of this enzyme on the several starches may be measured either by the soluble carbohydrates produced on a surplus of starch or by the residues left unattacked by a surplus of enzymes on a limited quantity of starch. Of the two, the latter method was selected because it seems more nearly to represent conditions of digestion as occurring *in vivo*. The following preliminary experiments were conducted in order to ascertain workable conditions:

Five hundred milligrams of the same starch was transferred quantitatively to 150-cc. Erlenmeyer flasks. The contents of each flask were shaken with 25 cc. of distilled water, after which they were placed in a thermostat at 37° C. After allowing sufficient time for the contents of the flasks to reach this temperature, such quantities of panase were added as are indicated in Table XII. At

the end of the time periods indicated in this table, the undissolved contents of the flasks were transferred quantitatively to previously weighed Gooch crucibles, washed, dried, and weighed as were the starch residues remaining after the action of malt extract. The results are given in Table XII.

TABLE XII

ACTION OF PANASE (COMMERCIAL PANCREATIC PREPARATION) ON LIMITED QUANTITIES OF RAW STARCH

Exp. No.	Wt. of starch used	Pancreatic preparation 1 cc. of	Time of digestion at 37° C.	Wt. of residual starch	Equivalent Wt. of starch hydrolyzed
	mg.	%	hr.	mg.	mg.
1	500	0.01	3	479.3	20.3
	500	0.01	3	471.1	28.9
2	500	0.1	3	477.1	22.9
	500	0.1	3	478.5	21.5

The results in Table XII are self-explanatory. They show that 1 cc. of a 0.1% solution of panase may be regarded as an excessive amount of enzyme for action on 500 mg. of starch, because it hydrolyzes essentially no more starch than does 1 cc. of a 0.01% solution in the same enzyme. The slightly discordant abnormal results in Experiment No. 1 are in all probability due to an experimental error.

In order to be assured of an excess of enzyme, 1 cc. of a 1% solution of panase was used in determining residues of raw starches remaining after the action of this enzyme preparation. The details of the technic were essentially the same as those of the technic employed in ascertaining residues of the same starches remaining after the action of malt diastase. The only modification was that 1 cc. of a 1% solution of the panase was used in place of 50 cc. of the malt extract, and that the temperature of the thermostat was changed to 37° C. Previous to the addition of the panase, the starch was suspended in 25 cc. of distilled water in order to supply a desirable volume. All other conditions of the test—time of digestion, method of filtration, etc.—were identical with those described under the preceding tests with malt extract. The averages of the results are recorded in Table XI.

Relative resistance of cooked starches to panase.—The data reported thus far in this study show clearly that raw starches differ in their resistance to enzyme action. The starches studied may be arranged in the order of their resistance as measured under different conditions. In this connection it seems advisable to as-

certain whether or not the order of their resistance to enzyme action in the cooked or gelatinized form was in any way related to any order of resistance in the raw condition, or to other data available on these starches. Accordingly, an examination was made of relative resistance to enzyme action of this group of starches in the cooked or gelatinized condition.

Stone (1897), O'Sullivan (1904), Day (1908), and Sherman, Walker, and Caldwell (1919), developed methods for estimating diastatic activity, using gelatinized starches as substrates. These methods are of two types. In the one, diastatic activity is measured by the time required for the disappearance of the starch as indicated by the starch-iodide test; in the other by the amount of reducing sugars produced by enzyme action under various arbitrary conditions.

It is possible that either of these two methods is applicable to the determination of resistance of cooked starches to amylolytic action. However, none of the technics employed in either method have been standardized or subjected to any critical or extensive study, so that no conclusions can be drawn regarding the reliability of the results which may be obtained by them. On the other hand, more or less contradictory views have been expressed concerning their relative merits.[2] For these reasons the writers decided to consider other possibilities.

Among these is a viscosimetric technic which Northrup and Hussey (1922-23) have developed for estimating proteolytic activity, using egg albumen or gelatin as substrates. More recently, Davison (1925) has modified this technic for estimating amylolytic activity of children's duodenal fluids or other enzymes, using Lintner's soluble starch as a substrate. In this technic, diastatic activity is measured by the time required to produce a certain percentage change in the initial viscosity of the substrate, which in this case is a gelatinized starch paste. That enzyme activity can be measured in this manner was demonstrated by Northrup and Hussey, who observed that the time required to produce a definite percentage change in the viscosity of a 3% gelatin solution was inversely proportional to the amount of trypsin employed. Davison observed the same relationship in the action of amylases on the viscosities of starch solutions. On the other hand, these investigators observed that the percentage of change effected in the initial viscosity of a substrate in a definite period of time is not proportional to the amount of enzymes employed.

[2] Personal communication from W. C. Davison.

It appears, therefore, that the principle of this technic is thoroly sound. It also has the advantage of simplicity and easy manipulation. For these reasons it was selected for this study. The apparatus[3] required for this technic consists of a number of Ostwald viscosimeters supported in a glass thermostat maintained at 34° C., in order that viscosities may be determined at this temperature.

The following preliminary experiment was performed and is inserted at this point in order that it may serve as a description of the details of the technic.

A 1% suspension of rice starch contained in an Erlenmeyer flask was gelatinized in a boiling water bath, then autoclaved for 15 minutes at 18 pounds pressure. The resulting "solution" was strained through muslin in order to remove any lumps. Ten cc. of this solution was transferred to each of the two Ostwald viscosimeters immersed in the thermostat. After allowing time for the starch solutions in the viscosimeters to acquire the temperature of the thermostat, the time required for 5 cc. of the solutions to flow through the capillary of the viscosimeters was observed. This observation was repeated several times in order to ascertain the stability of the viscosity, which is essential in this technic. This value, if found to be constant, is recorded, in seconds, as the initial viscosity of the solution. Starch solutions thus prepared are almost always constant in viscosity, but occasionally the viscosity will decrease with each successive determination. Such a solution must be discarded. After the viscosity of the solution was found to be stable, 0.1 cc. of 0.01% panase solution was quickly and thoroly mixed with the contents of the viscosimeter and the time of adding was recorded. Viscosity determinations were then made at carefully noted time intervals, usually four minutes, and continued until the viscosity had decreased to 80% or less of the initial viscosity, which decrease must take place within two hours. The initial viscosity was placed at 100. All of the succeeding readings were converted to the 100 basis by multiplying each by 100 and dividing the product by the initial reading of the "solution." An illustration will make this procedure clear. The initial viscosity of this 1% rice starch solution was found to be 144 (seconds required for 5 cc. to flow through the capillary tube). This value was placed equal to 100. The viscosity 12 minutes later (in terms of seconds required for the 5 cc. to

3 The authors are indebted to Dr. Wilburt C. Davison, of the Department of Pediatrics, Johns Hopkins University, for the use of his laboratory facilities in carrying out these viscosity determinations and for valuable guidance in this part of the investigation.

flow through the capillary) was observed to be 118.6 seconds. On the 100 basis this would equal $\frac{100 \times 118.6}{144} = 82.3$. This method of recording results is necessary in order that viscosities and decreases in viscosities observed by means of different viscosimeters may be reduced to the same scale. This entire procedure was repeated on another 10-cc. portion of the same paste, using 0.2 cc. of a 0.01% solution of the same panase solution. The results are recorded in Table XIII. On Plate 2 viscosities have been plotted on the vertical axis against time intervals elapsing between each determination on the horizontal axis.

TABLE XIII

RELATIONSHIP OF PANASE CONCENTRATION TO TIME REQUIRED FOR PRODUCING A GIVEN DECREASE IN THE VISCOSITY OF A 1% PASTE OF RICE STARCH

0.01% Panase used, cc.			
0.1 cc.		0.2 cc.	
Time	Viscosity	Time	Viscosity
Min.	%	Min.	%
0.0	100.0	0.0	100.0
2.0	93.95	2.25	92.2
6.0	88.4	4.75	85.2
9.0	84.7	7.9	80.0
12.0	82.3	11.0	76.0
16.0	78.47	13.7	72.8
20.0	75.0	....	....
23.0	72.9	....	....
25.7	70.8	....	....

These preliminary experiments were performed primarily for the purpose of ascertaining satisfactory conditions for subsequent experiments. Incidentally, they also served to confirm the previous observations of Northrop and Hussey (1922-23) and Davison (1925) that there is direct proportionality between concentration of enzymes and time required to produce a definite percentage of decrease in the initial viscosities of the substrate.

The above technic was applied to the eleven starches recorded in Table III in order to ascertain their relative resistances in the cooked or gelatinized condition. The work was carried out as follows:

One per cent solutions of these several starches (except the potato, of which a 0.25% solution was used) were prepared simultaneously as described in the above preliminary experiment on rice starch, that is, 1% suspensions were gelatinized in the water bath, and then autoclaved. In each solution the initial viscosity and its successive decreases at regular time intervals after the addition of a 0.1-cc. portion of a 0.01% panase solution, were determined

and recorded as described in the preceding preliminary experiment on rice starch. As the thermostat used had a capacity for only four viscosimeters, no more starch solutions could be worked with simultaneously; therefore the eleven starches had to be divided into three groups. However, all groups were taken care of on the same day, so that the same enzyme solution was used for all in order that the results might be comparable. Duplicate results were obtained by repeating the entire work with fresh solutions on a following day. The results are recorded in Table XIV.

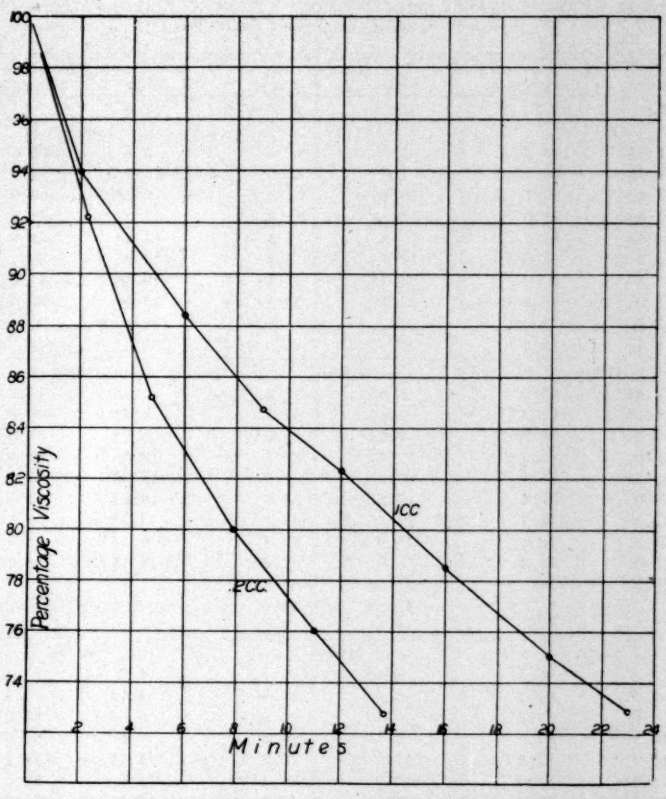

Fig. 2. Action of 0.01% Solution of Panase on Gelatinized Rice Starch (1% Paste)

The data contained in Table XIV have been used for the calculation of the values contained in Table XV. Column 1 of Table XV contains the time periods required for the solutions of the several starches to decrease in viscosity to 80% of their respective initial viscosity. Column 2 contains these time values converted to the 100 basis by multiplying the values of Column 1 by 100 and

dividing this product by the longest time period, that of No. 11954, which is 32.0 minutes. The values in Column 3 show the relative extent of hydrolysis of the several starches. These values are the differences between 100 and the corresponding values of Column 2. The data recorded in Tables XIV and XV are plotted graphically on Plate 3.

This concludes the experimental data accumulated in this study.

TABLE XIV

ACTIONS OF 0.1 CC. OF 0.01% PANASE "SOLUTION" ON 10 CC. STARCH PASTE AT 34° C.
(Viscosity Readings Converted to 100 Basis)

Time	Decrease in viscosity	Time	Decrease in viscosity	Time	Decrease in viscosity
Min.	%	Min.	%	Min.	%
11717		11719		11800	
1.83	93.2	3.7	91.9	1.8	96.6
4.33	90.23	7.0	88.6	5.0	92.5
8.66	86.7	10.16	84.8	8.23	89.9
10.66	85.0	13.2	82.5	11.5	86.9
14.66	82.7	17.3	78.8	14.16	85.0
16.5	81.8	19.75	77.4	16.66	83.4
18.5	80.9	23.33	75.4	19.66	81.6
21.0	80.0	27.1	73.5	23.0	80.0
23.8	78.4	.....	.....	28.0	77.9
26.5	77.5	.....	.....	32.0	76.7
11804		11954		11948	
2.83	95.7	2.63	97.4	1.25	94.2
6.16	92.2	6.66	93.7	4.33	90.6
9.2	88.8	9.83	91.0	8.33	87.0
12.5	86.2	12.4	88.7	10.5	85.6
15.75	83.1	16.4	87.2	15.83	82.9
19.0	80.4	19.5	85.1	19.83	81.1
22.33	78.7	22.75	83.3	23.83	79.3
25.0	76.8	25.9	82.3	26.33	78.9
28.5	75.4	28.9	81.3	28.16	78.6
30.33	74.1	31.33	80.5	.....	.....
.....	.....	35.33	79.4	.....	.....
.....	.....	37.33	78.7	.....	.....
11901		11904		Maize	
2.2	96.2	2.16	91.3	1.7	93.5
5.5	91.2	5.2	85.8	4.16	90.4
7.6	89.2	7.5	82.6	6.66	87.0
10.5	86.4	9.75	80.0	9.5	84.0
13.7	83.8	12.0	78.2	11.75	82.7
16.83	82.0	14.0	76.4	13.5	81.7
19.2	79.6	18.0	72.4	15.9	79.9
26.16	76.0	.....	.....	17.9	79.1
.....	.....	.....	.....	22.25	76.9
.....	.....	.....	.....	24.25	77.2
Irish Potato				Rice	
1.25	86.6			2.4	94.6
3.83	79.4			6.2	88.3
6.33	76.9			9.4	83.8
8.5	74.9			13.3	80.1
10.5	73.8			16.1	77.59
12.5	73.6			18.9	75.2
.....	.....			22.4	72.2

TABLE XV

RELATIVE DECREASE OF VISCOSITY OF ONE PER CENT STARCH PASTES AS CAUSED BY PANASE

Purified starches	Class	Source	No. 1	No. 2	No. 3
			Min.	Min.	Min.
11717	H. R. W. Wheat	Neb.	19.7	61.56	38.44
11719	H. R. W. Wheat	Neb.	15.9	49.68	50.32
11800	H. R. S. Wheat	No. Dak.	23.0	72.06	27.94
11804	H. R. S. Wheat	No. Dak.	19.0	62.18	37.82
11954	H. R. W. Wheat	Montana	32.0	100.00	0.00
11948	H. R. W. Wheat	Montana	22.8	71.25	28.75
11901	H. R. W. Wheat	No. Dak.	18.8	58.75	41.25
11904	H. R. W. Wheat	No. Dak.	10.1	31.56	68.44
Maize, commercial			16.4	51.25	48.75
Irish potato, commercial			3.7	11.59	88.41
Polished rice, commercial			13.1	40.94	59.06

Col. 1. Time required for viscosity to fall to 80% of the initial viscosity.
Col. 2. Time values recorded in Column 1 converted to the 100 basis.
Col. 3. Extent of hydrolysis as indicated by the differences between 100 and the corresponding values contained in Column 2.

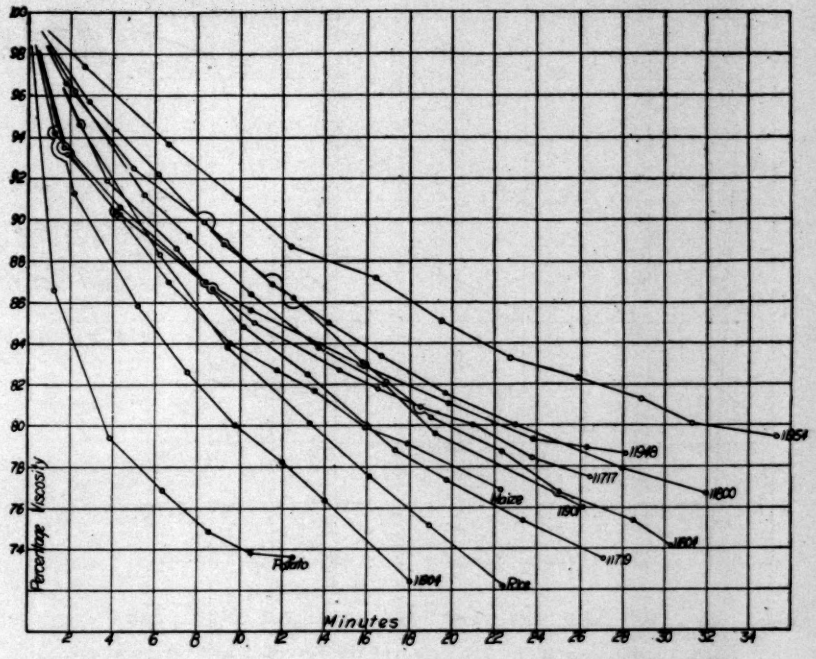

Fig. 3. Decreases in the Viscosity of Starch Pastes by the Action of Panase

Discussion and Interpretation of Results

All starches examined in this study showed individualities in all the comparative tests applied. This deportment accords with previous observations of Meyers (1895), MacNider (1912), Langworthy and Devel (1920-22), Collatz (1922), Rask and Alsberg (1924), and others, who have already discussed variations among starches

as revealed by some one single technic. A further insight into the nature of these variations can be gained most readily from an examination or comparison of the results obtained by the several technics employed in this study and contained in the preceding tables. These have been assembled on Plate 4. On this plate the

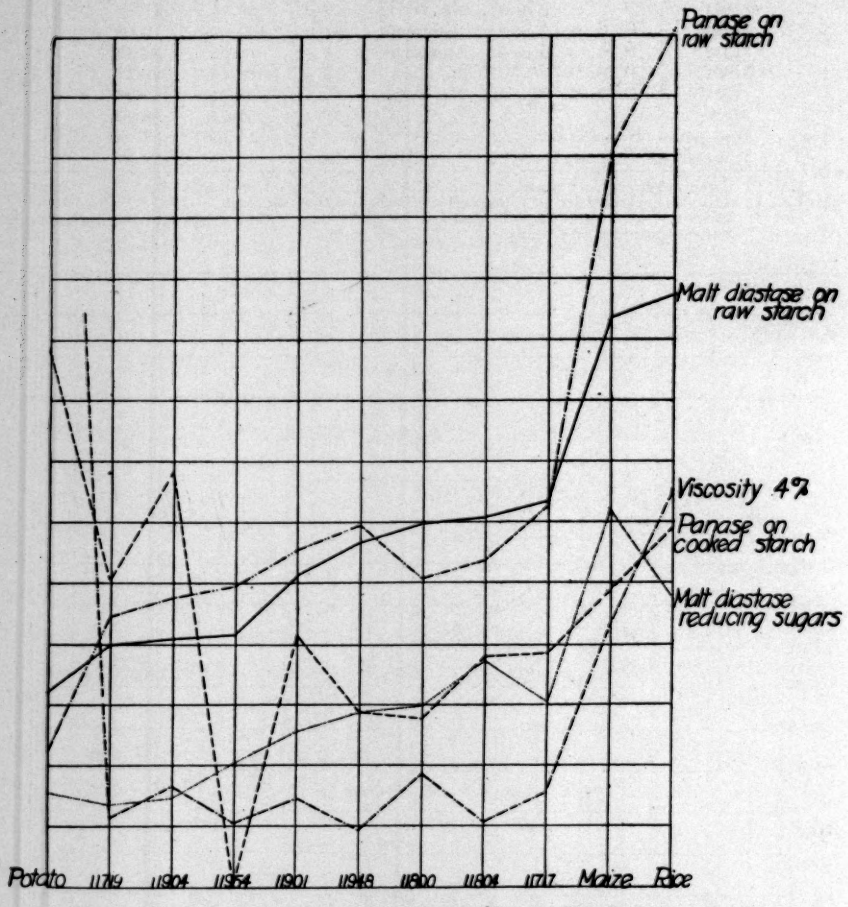

Fig 4. Correlations of the Properties of the Several Starch Preparations.

starches have been arranged in the order of their resistance in the raw form to malt diastase as measured by the amount of starch rendered soluble.

Attention may first be directed to the courses of the curves which show the extent of hydrolysis of the raw starches by panase and by malt extract. It will be noticed that there is a general parallelism between these two curves throughout their entire

courses. Only one starch can be regarded as distorting this parallelism. This close parallelism is strongly indicative of a correspondingly close similarity in the actions of malt diatase and pancreatic amylase. The persistance of this parallelism in the midst of the other curves which show no parallelism indicates, furthermore, that the similarity in the actions of these two enzymes is of a fundamental nature. Similarity in the actions of two amylolitic enzymes of such widely different origin suggests a similarity in the mechanism of all amylolitic actions.

Conclusions of a different nature may be drawn from a study of the three curves showing the extent of enzyme action on starches in the raw or ungelatinized condition. Curves 1 and 2 show amounts of starch hydrolyzed as results of the action of surplus quantities of enzymes on limited quantities of starch. Curve 5 shows quantities of starch hydrolyzed as results of the action of limited quantities of enzymes on excessive quantities of starches. Attention has already been called to the parallelism of the two curves showing the quantities of starch hydrolyzed by excessive amounts of enzyme. However, the curve showing the amounts of starch hydrolyzed under the latter condition, i.e., by deficient amounts of enzymes, follows a somewhat different course. Accordingly, the hydrolysis products produced under the latter condition must represent something else than that represented by the hydrolysis products produced under the former condition. It seems quite obvious that the hydrolysis products which are the result of the action of surplus quantities of enzymes on limited quantities of starch, represent portions of the starch grains which are susceptible to hydrolysis under the conditions of these experiments. These portions probably correspond to that portion of the starch grain which Meyer (1895) designated as B amylose. However different conditions prevail and correspondingly different results may therefore be expected where limited quantities of enzymes are applied to surplus quantities of starch, as was the case in the technic in which tungstic acid was used as the inhibitory reagent and in which the extent of hydrolysis was measured by the amount of reducing sugars produced. Under these conditions there may be such a large surplus of starch present that only the starch portions which are most readily hydrolyzed will adsorb or absorb all the enzyme present, which is relatively little. Other portions or areas of the starch grain which might also be hydrolyzed under those conditions, tho with less ease or less rapidity, may remain untouched because no enzyme is available for them. If this is

true, then the results obtained under the latter condition are indicative of the ease of hydrolysis of those portions of the starch grains which are selected for action and not a measure of the proportion or quantity of the entire grain which can be hydrolyzed under the conditions of the experiment. This course of reasoning therefore leads to the conclusion that the former technic measures the amount of that portion of starch grain which is capable of hydrolysis under such conditions, whereas the latter technic measures the ease with which this portion is hydrolyzed. Accordingly, it seems probable that amylolitic resistance can be resolved into at least two factors: a quantitative factor, denoting the amounts of starch susceptible to hydrolysis under definite conditions; and a qualitative factor, denoting the ease with which that portion may be hydrolyzed.

There is very little parallelism between the viscosities of the starch pastes and their digestibilities in the cooked condition. The only notable exception is the potato starch. This produces by far the most viscous paste and is also the most readily hydrolyzed or digested in the gelatinized condition. However, in the potato starch, viscosities were in all probability recorded on pastes composed of unbroken or unruptured grains, whereas enzyme action must have taken place on ruptured grains, for autoclaving at 18 pounds pressure undoubtedly ruptured the grains of this starch which were observed to rupture when heated only to 90° at atmospheric pressure. On the other hand, the grains of the other starches remained intact under these conditions, as shown by microscopic examinations after staining with congo red. Because of these facts, the greater digestibility of the gelatinized potato starch may be regarded as due to the ruptured condition of its grains, and its high viscosities are suggestive of grains which have swollen to a point at which rupturing is inevitable.

The curves on Plate 4 indicate a slightly greater parallelism between amylolitic susceptibility of starches in the raw, or ungelatinized, state and the viscosities of their pastes. The viscosity of a starch paste is determined largely by the relative volume represented by the swollen grains in the paste. That is obvious from the viscosity equation: $\log y = mx + \log b$, which has been found to hold in the case of starch pastes. This volume, in turn, is determined by the extent to which the individual grains have swollen. Alsberg (1926) has pointed out that three factors may be operative in determining the extent to which a starch granule will swell. These are: (1) its rigidity, or the ease with which

TABLE XVI

DATA OBTAINED ON KHARKOV AND MINTURKI STARCHES

Sample	Source	Viscosity in centipoise at 3.5%	Temperature.	Precipitation	Temperature divided by rainfall	Extent of hydrolysis in raw condition by		Extent of hydrolysis in cooked condition	b/m	b	m
						Malt extract	Panase				
			°C.	In.		%	%	%			
KHARKOV											
11901	No. Dak.	45.57	63.73	1.60	39.8	51.1	55.9	41.25	2.2	0.74	0.326
11948	Mont.	58.83	60.76	1.89	32.15	56.7	59.85	28.75	0.12	0.07	0.536
11717	Neb.	84.85	70.6	2.84	23.25	63.1	62.7	38.44	0.358	0.17	0.488
MINTURKI									m/b	b	m
11904	No. Dak.	89.98	63.73	1.60	39.8	59.84	47.55	68.44	7.14	0.19	0.4849
11954	Mont.	60.79	60.76	1.89	32.15	80.32	49.5	0.0	6.28	0.083	0.521
11719	Neb.	73.2	70.6	2.84	23.25	53.2	44.95	15.9	2.5	0.08	0.5425

its anatomical structure is softened by moist heat; (2) the inherent swelling power of the granule substance; and (3) the relation of mass of swelling substance to surface area of the granule. As swelling of starch grains determines their volume in a paste, the above factors must be operative in determining viscosities. Furthermore, they may function in this manner either jointly or separately or in different orders of importance. Amylolitic susceptibility of starches may be due to the same factors or a similar group of factors. In that case the partial relationship between enzyme susceptibility of starches and the viscosity of their pastes may be explained by the assumption that in each individual starch the above factors arrange themselves in a certain order of importance in determining its amylolitic susceptibility, and in a different order in determining the viscosity of its paste.

The same course of reasoning may explain the partial relationships between other properties of starches.

The preceding tables have been examined for other data of possible significance. These have been assembled in Table XVI.

An examination of the data on Kharkov starches shows the existence of the following relationships and correlations (in the case of all three samples):

1. The data on viscosity of pastes at 5.5% rainfall during maturing periods, and extent of hydrolysis in the raw condition by means of both malt diastase and pancreatic amylase, parallel or vary directly with one another; that is, the data on these constants or properties increase or decrease with one another.

2. Temperature divided by rainfall varies inversely with all the values referred to in statement No. 1.

3. The data representing the values b/m, b, and extents of hydrolysis in the cooked condition, parallel or vary directly with one another.

4. The values representing m vary inversely with the values referred to in statement No. 3.

An examination of the data on Minturki starches shows the existence of the following relationships and correlations (in the case of all three samples):

1. The data on viscosities of pastes at 5.5% and extent of hydrolysis in the cooked condition by pancreatic amylase, parallel or vary directly with one another.

2. The values representing ratios of temperature divided by rainfall and m divided by b parallel or vary directly with one another.

3. The values for m vary inversely with the values referred to in statement No. 2.

4. The values for m and the rainfall during the thirty days preceding harvest are parallel; that is, the correlation is direct.

Particularly striking and possibly also significant are the relationships between geographical localities of growth and the variable extents of hydrolysis of starches in the cooked condition. When the starches of either variety are arranged in the order of the extent of their hydrolysis in the cooked condition, it will be noticed that the starches produced in North Dakota are the most readily hydrolyzed, and those produced in Montana are the least readily hydrolyzed, whereas those produced in Nebraska are between the two. Whether all wheat starches produced in these localities or states would arrange themselves in this order of digestibility is an interesting question which can be answered only by the examination of more samples. However, these data suggest the possibility that geographical locality of growth may have a distinct influence on the digestibility of starches. Due allowance must be made for the relatively small number of observations of the preceding correlations. Accordingly, they can be regarded as only suggestive of influences which the different attributes and properties of starches may exert on one another. These data do show, however, that **the variable individualities of starches are due to several more or less interdependent factors, several of which must be determined in order to establish the identity of any one starch.**

Conclusions

The experimental data reported in this paper suggest the following conclusions:

1. Starches, both in the raw and in the gelatinized condition, vary in amylolitic susceptibility independently of their botanical origin.

2. There is no relationship between amylolitic susceptibility in the raw and in the gelatinized conditions.

3. The mechanism of amylolitic action is similar, regardless of the origin of enzymes and substrates.

4. Amylolitic susceptibility of starches can be resolved into qualitative and quantitative factors.

5. Amylolitic susceptibility of starches varies to a partial extent with the viscosity of their pastes.

6. Variable individualities of starches are due to several variable factors (possibly three) which arrange themselves in different orders of importance in determining the several variable chemical and physical properties of starches, and thereby prevent parallelisms among such properties in a series or group of starches.

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RELATION OF CRUDE PROTEIN CONTENT OF FLOUR TO LOAF VOLUME [1]

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Introduction

The crude protein content of wheat and flour has become increasingly significant in the merchandising of the hard spring and hard winter wheat crops. A large proportion of the hard wheat marketed in the United States is subjected to chemical analysis before the price is determined. Certain lots of the lower grades of wheat have not infrequently sold for higher prices than the minimum of the high or No. 1 grade because of their high protein content. While premiums paid for high protein wheat have been quite variable, being negatively correlated with the average protein content of the crop, attention is now given to the percentage of protein even in seasons of high average percentages of that constituent.

The correlation between protein content of wheat and baking value of flour has accordingly become of interest to all branches of the wheat trade. Dealers in wheat who probably never thought of its composition until a few seasons ago, now offer their choice wheat with a chemist's certificate very prominently displayed.

Several extensive studies of the relation of wheat composition to baking quality have been reported in the recent literature. In the majority of instances a positive correlation has been detected between the percentage of protein (or gluten) and bread making value. It has been generally recognized that other factors are operative in influencing the quality of bread that can be produced. "Quality" of gluten has been referred to frequently, but as yet no convenient and accurate test has been devised which results in a numerical value for expressing degree of quality. Diastatic activity of flour is doubtless an important factor, but this is not so easily determined as some of the other properties. The result has been that the percentage of crude protein is about the only constituent, or property, that has been commonly accepted as an index of baking value. It can be determined quickly, and with a fair degree of precision, several analysts usually reporting values for the

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same sample which vary within relatively narrow limits. A uniform basis for reporting the results has been adopted, to the extent that the percentage of nitrogen in the wheat or flour multiplied by the factor 5.7 is reported as the percentage of crude protein. The suggestion has occasionally been advanced that milling and baking tests of each lot or parcel of wheat should be made. Those familiar with the labor and expense involved in making such tests, the extensive facilities which would be necessary in handling the work in a large terminal market such as Minneapolis, and the number of replicates necessary to secure true values, recognize the impracticability of such a procedure in thus arriving at the market value of milling wheat.

Protein content of wheat or wheat flour has been correlated with loaf volume of bread baked from it by several investigators. Zinn (1923) computed the coefficients of correlation between loaf volume and crude protein content of wheat and wheat flour from data published by various state experiment stations. Minnesota commercial varieties of spring wheat showed a coefficient of $+ 0.1827 \pm 0.0459$ for protein in wheat and loaf volume. Pure strains of Minnesota wheat gave a higher positive correlation, expressed by the coefficient $+ 0.4621 \pm 0.0766$. North Dakota spring wheat gave a negative correlation, $- 0.1172 \pm 0.0588$, while Montana spring wheat showed $+ 0.3555 \pm 0.1011$.

Upon computing the correlation coefficient between protein in flour and loaf volume, Zinn found a positive correlation in every flour except that from North Dakota spring wheat. This exception has been explained by Mangels (1926), as noted later in this paper. Minnesota spring wheat flour gave a coefficient of $+ 0.2586 \pm 0.0442$ for commercial wheat varieties, and $+ 0.5469 \pm 0.0689$ for pure strains of wheat. With a few exceptions, Zinn recorded a higher degree of correlation between protein in flour and loaf volume than between protein in wheat and loaf volume.

North Dakota wheats of three crop years, 1921, 1922, 1923, were studied by Mangels and Sanderson (1925), who found a positive correlation between protein content and loaf volume for each of these seasons. The negative correlation previously noted by Zinn did not appear when the data were segregated by crop seasons instead of including them in a single group. They found for 1921, $+ 0.307 \pm 0.053$; for 1922, $+ 0.427 \pm 0.047$; for 1923, $+ 0.345 \pm 0.043$.

In a study of Nebraska flours, Blish and Sandstedt (1925) reported a positive correlation between loaf volume and protein content of wheat, $+ 0.304 \pm 0.058$.

Mangels (1926) studied further the reports of North Dakota wheat crops, and using the data of eleven crop years computed the coefficients of correlation between protein content of spring wheat flour and loaf volume. He offered an explanation of the negative correlation for North Dakota wheats reported by Zinn (1923). When the durum wheat samples were included with the hard red spring wheat samples from the crops of 1908, 1909, and 1910, the same negative correlation reported by Zinn was found. When the durum samples were omitted, the hard red spring samples showed a positive correlation of high order, $+0.523 \pm 0.062$. The results of the entire series computed by crop years showed a positive correlation for eight of eleven years. Of the eight the lowest coefficient was $+0.307 \pm 0.053$, and the highest $+0.547 \pm 0.032$.

Grewe (1926) subjected flours milled from hard and soft types of American wheats to baking tests. The coefficient of correlation between loaf volume and the protein content of these flours was $+0.6781 \pm 0.0911$.

Supplementing the report of Bailey (1924), the results of protein determinations and test bakes made in the Minnesota State Testing Mill have been studied for the crop years 1921-25 inclusive. The samples of wheat included in this study were all of the hard red spring type, representing all grades of hard red spring from No. 1 to No. 5. The 266 lots of wheat originated in the four northwest states—Minnesota, North Dakota, South Dakota, and Montana approximately 80 per cent being grown in Minnesota. Nearly all the lots were purchased in carload quantities in the Minneapolis market. Milling tests of each lot were made, using 100 bushels for each of duplicate tests, following the procedure outlined by Bailey (1923). Straight grade flour was produced in each case. Test bakes were made of the flour produced in each milling test, and the results of the replicated baking tests of each lot of wheat were averaged. The baking procedure described by Bailey (1923) was used throughout the entire period. The formula follows:

	grams	Per cent
Flour	350	100.
Yeast	10.5	3.0
Sugar	10.5	3.0
Salt	5.25	1.5
Lard	5.25	1.5
Water	Sufficient	

The doughs were mixed in a mechanical mixer, fermented at 30° C., proofed at 34° C., and baked at about 215° C. Loaf volume was determined in the loaf volume tester of the Industrial Appliance Company.

Protein determinations of the straight grade flours were made in duplicate by the Kjeldahl method, approved by the A. O. A. C.

The coefficient of correlation of loaf volume with crude protein content of straight grade flour milled from hard red spring commerical wheat of four crop seasons and part of the fifth season was found to be $+ 0.2711 \pm 0.038$. This is a significant positive correlation. It is of a lower order than that reported by several investigators for pure strains of wheat, but of a higher order than that reported by Zinn (1923) for commercial wheats of Minnesota. The coefficient is somewhat lower than the mean of the coefficients + 0.310, reported by Mangels (1926) for eleven crop years.

Function of Protein Content in Increasing Loaf Volume

The volume, or cubical displacement, of test loaves baked under controlled conditions is the most commonly accepted single criterion of the baking strength of flour. It is the one physical characteristic of a loaf of bread that can be measured with instruments and the resulting measurement recorded in numerical values. In the computations which follow it will be used as one factor, and its relation to protein content will be traced. The percentage of protein will be that of the straight grade flour, rather than of the wheat.

Data recorded in the literature have already indicated that when the same baking formula is used and a uniform treatment accorded each dough (with the possible exception of fermentation period), the loaf volume does not increase regularly with increasing protein content. Bailey (1913) observed that an increase of 1% of crude protein between 9.5 and 10.5% effected an increase in loaf volume of 6.6%, while an increase of 1% of crude protein between 13.5, and 14.5% effected an increase in loaf volume of only 0.8%. These were the averages of relatively large numbers of flour samples, grouped on the basis of protein content with a range of 1% of crude protein in each group.

Thomas (1917) and Stockham (1920) noted a similar behavior of flours milled from hard spring wheat until 15% of protein was reached. An increase above 15% was accompanied by a diminished loaf volume.

Shollenberger (1923) grouped the flour samples examined by him on the basis of the protein content of the wheat from which they were milled. A range of 2% of crude protein was represented in each group. With the hard spring wheat flours, the average loaf volume (by groups) increased with increasing protein content to 15.9% of crude protein. Further increases were accompanied by a diminished loaf volume. With the hard winter wheats, the loaf volume increased with the protein content until the latter reached 11.9%. Thereafter the loaf volume

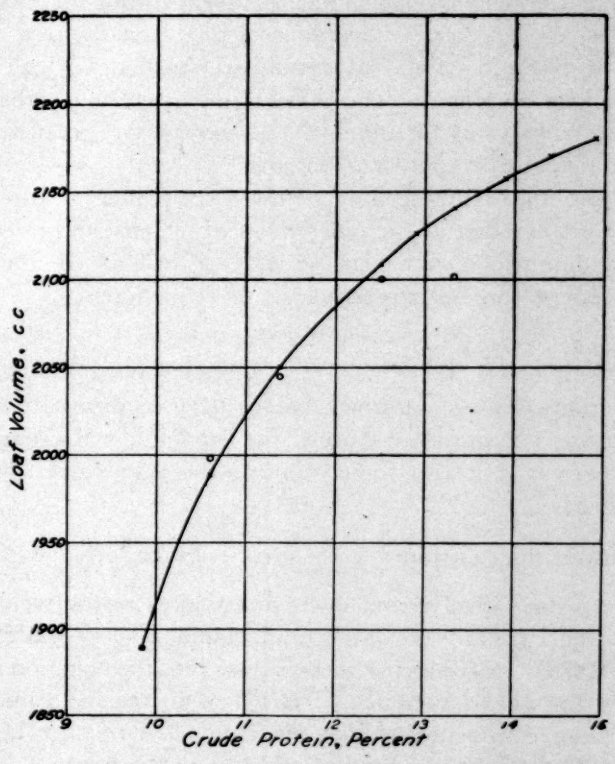
remained practically stationary (excepting one group) with increasing protein content. This is shown by the data in Table I.

TABLE I

RELATION OF PROTEIN CONTENT OF WHEAT TO VOLUME OF LOAF, AS REPORTED BY SHOLLENBERGER (1923)

Protein range in wheat.	Loaf volume	
	Hard red spring wheat flour	Hard red winter wheat flour
%	cc.	cc.
7.9 and lower	1760	1880
8.0 — 9.9	2136	2081
10.0 — 11.9	2176	2158
12.0 — 13.9	2249	2157
14.0 — 15.9	2287	2134
16.0 — 17.9	2252	2110
18 and higher	2198	2140

The 266 flour samples milled from as many different lots of wheat at the State Testing Mill constitute excellent material with which to measure the function of crude protein in increasing loaf volume. The data resulting from the analysis and baking tests of these samples were accordingly arranged in groups on the basis of protein content—all the samples which contained less than 10% of crude protein were



Relation of Loaf Volume to Crude Protein Content of Hard Spring Wheat Flour

included in the first group, those with 10 to 10.99% in the second group, and so on by 1% intervals to 13.99%. The relatively few samples which contained more than 14% of crude protein were included in the sixth group. The average loaf volume of the samples in each group was then calculated. Relation of protein content to loaf volume is shown by these data, which are recorded in Table II.

TABLE II

AVERAGE PROTEIN CONTENT AND AVERAGE LOAF VOLUME OF FLOURS ARRANGED IN GROUPS ON THE BASIS OF PROTEIN CONTENT

Crude protein		
Range	Average	Average loaf volume
%	%	cc.
Less than 10	9.85	1889
10 — 10.99	10.60	1998
11 — 11.99	11.38	2045
12 — 12.99	12.46	2100
13 — 13.99	13.32	2102
Over 14	14.90	2230

The same data are recorded graphically by means of the points or small circles in the figure. It is evident from the position of these points that they do not fall on a smooth curve. Their deviation from the anticipated position is probably due to the operation of two factors: (1) the relatively large experimental error of the baking test, and (2) a deficiency in diastatic activity of certain of the flours which contain a large percentage of gluten. The two groups averaging highest in protein content, namely, 13.32, and 14.90%, showed the greatest deviation from the anticipated average loaf volume.

An inspection of the position of these six points indicates that the curve is hyperbolic, that is, each increment of increase in protein content results in a diminished increment of increase in loaf volume. Such a hpyerbolic curve can be expressed by the equation:

$$\text{(loaf volume) } y = \frac{x}{a + bx} + c.$$

The calculation of the co-ordinate values of y in terms of x (protein content) affords a means not only for smoothing the curve into a regular hyperbola, but also for computing the values of y for any desired value of x.

Assuming the relation, $\frac{x - x_k}{y - y_k} = a + bx$, in which x_k and y_k are the smallest values involved on the x and y axes respectively, we have solved for the constants a and b, and found $a = -0.0159112$, and $b = +0.0022297$. Introducing these values into the equation last given, and solving for y(loaf volume) in terms of increasing values of x, a curve has been constructed which represents the relation between the two variables, and is shown by the solid line in the figure.

Such a calculation, involving the use of the constants a and b, and with 12.46 as the value for x, would be as follows:

$$\frac{12.46 - 9.85}{y - 1889} = -0.0159112 + (0.0022297)\ (12.46)$$

$$\frac{2.61}{y - 1889} = -0.0159112 + 0.0277821 = 0.0118709$$

$$\frac{2.61}{0.0118709} = y - 1889$$

$$219.9 = y - 1889, \text{ and } 1889 + 219.9 = y$$

$$\text{or } y = 2108.9.$$

In a similar manner any value for y in terms of x may be computed. Such a series of values was calculated, and subsequently used in locating the co-ordinate points for the construction of the curve represented by the solid line in the figure. The values are recorded in Table III.

TABLE III

COMPUTED VALUES OF Y (LOAF VOLUME) IN TERMS OF X (CRUDE PROTEIN)

Crude protein (x)	Loaf volume (y)
%	cc.
9.85	1889
10.60	1986
11.38	2051
12.46	2109
12.90	2126
13.32	2141
13.90	2157
14.40	2170
14.90	2180

A study of the graph, and of the formula which represents its mathematical expression, shows that each unit increase in protein content is accompanied by a progressively smaller increase in loaf volume as the curve is traversed from the lower to the upper limits of percentage of protein. This means that the significance of each unit difference in loaf volume must be interpreted in terms of the method of baking, and the usual range in loaf volume for the method. Thus, a baking test method involving large dosages of yeast may result in loaves, two-thirds of which vary between 2400 and 3000 cc. In such instances, an increase of 100 cc. in loaf volume between 2400 and 2500 cc. might result from a relatively small increase in protein content. On the other hand, if the baking test method yields loaves, two thirds of which range between 2000 and 2500 cc., then a gain of 100 cc. from 2400 to 2500 cc. is made only with a substantial increase in protein content.

It is also evident that the results of test bakes must be similarly interpreted in guiding blending practices. It is probable, as deduced

from the mathematics involved, that when two flours are under consideration, both of which yield loaves of relatively large volume for the testing method, the difference in loaf volume is more significant in terms of their probable contribution to the blend than are like differences when the loaves are smaller in volume.

Summary

Crude protein content of straight grade flour milled from hard spring wheat in the Minnesota State Testing Mill was found to be positively correlated with the loaf volume of bread baked from the flour. In the instance of 266 such samples produced during a period of five crop seasons, the coefficient of correlation was $+ 0.271 \pm 0.038$.

Increase in loaf volume with each unit increment of increase in protein content diminishes with increasing percentages of protein. A plot of loaf volume as ordinates (y) with protein content as abscissas (v) results in a hyperbolic curve. This hyperbolic curve can be expressed by the equation: $y = \frac{x}{a + bx} + c$. Assuming the relation $\frac{x - x_k}{y - y_k} = a + bx$, we have solved for the constants a, and b, and found $a = - 0.015112$, and $b = + 0.0022297$. Introducing these values into the equation above and solving for y (loaf volume) in terms of increasing values of x, a smooth curve has been constructed which represents the relation between the two variables.

Altho each unit of increase in protein content in the high protein material is not accompanied by a large increase in loaf volume, this does not imply that the differences in loaf volume noted are not of significance and value. It cannot be assumed that a unit increase in loaf volume throughout the range encountered when a single baking method is followed will represent a unit of value. Each unit of increase in loaf volume as the higher levels are approached represents a greater value than at the lower levels, and should contribute more to a blend or mixture than a like unit at the lower levels for the testing method that is being employed.

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WHEAT AND FLOUR STUDIES VIII
THE COMPOSITION OF WHEAT AND MILL PRODUCTS FROM FROZEN AND NON-FROZEN WHEAT HARVESTED AT VARIOUS STAGES OF MATURITY [1]

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This paper is a continuation of the investigations previously reported by Sharp and Elmer (1924), Sharp (1924, 1925) and Whitcomb and Sharp (1925, 1926) on the effect of freezing on the wheat kernel. A description of the samples used in this work, and data in regard to their composition may be found in the papers by Sharp (1925), and Whitcomb and Sharp (1925, 1926) and therefore will not be repeated.

The mill products obtained in the first milling of this series of wheat samples, as given in Table IV of the paper by Whitcomb and Sharp (1926), were analyzed for crude protein and ash. The results of the protein determinations are given in Table I. Considering the data as a whole, there is a slight tendency for the mill products as well as the wheat to increase in protein content as the kernel develops. In the immature stages of development the frozen wheat contained less protein than the non-frozen wheat. This difference continues up to the samples collected on the 27th day. From that point on, with the exception of the samples collected the 41st day, the protein content of the frozen wheat agrees very well with that of the non-frozen.

In a later paper an explanation will be offered for a part of this difference in protein content between the frozen and non-frozen samples. There is a tendency toward a more equal distribution of the protein between the various mill products at the early stages of kernel development. The flour milled from the frozen samples contained as much protein as the original wheat, while the bran contained less. It is very difficult to get a good separation of mill streams when milling such immature wheat, so the incompleteness of the separation in milling may account for some of the differences in composition when the mill streams are compared with those from normal wheat. The differences are probably not all accountable to errors in milling, for we find that the bran from

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the frozen immature samples actually contains less protein than the original wheat, while the bran from the non-frozen samples contains slightly more. We observe that beginning with the samples collected on 27th day, when the wheat contained about 46 per cent moisture at the time of freezing, and for all more mature samples, the protein content of the mill streams shows little effect of freezing.

TABLE I

PROTEIN CONTENT OF WHEAT AND MILL PRODUCTS (DRY BASIS) MILLED FROM FROZEN (F) AND NON-FROZEN (NF) WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY.

Lab. No.	Approx. age of kernel	Crude Protein (N× 5.7)				
		Wheat	Flour	Shorts	Bran	Scourings
	Days	%	%	%	%	%
131 NF	13	16.13	15.07	16.86	16.78	.....
132 F	13	14.59	14.60	15.31	13.70	.....
133 NF	17	15.02	14.39	16.40	16.14	.....
134 F	17	14.49	14.47	15.50	13.19	.....
135 NF	21	15.40	14.62	16.90	16.60	13.77
136 F	21	15.19	15.27	16.84	14.80	15.33
137 NF	25	15.77	15.10	18.18	18.15	14.30
138 F	25	15.10	14.47	17.07	15.84	15.60
139 NF	27	16.19	15.62	18.28	17.73	13.51
140 F	27	16.18	15.47	19.12	17.89	11.86
141 NF	29	17.00	15.91	19.05	19.20	14.00
142 F	29	17.29	17.14	20.50	18.66	12.36
145*NF	33	16.73	16.08	18.87	19.09	13.80
146 F	33	17.08	16.37	19.78	19.64	12.00
147 NF	35	17.08	16.39	18.75	19.34	14.30
148 F	35	16.70	16.07	19.48	18.47	11.30
149 NF	38	16.98	16.42	19.57	19.22	12.84
150 F	38	17.06	16.09	20.40	20.02	12.55
151 NF	41	17.81	16.47	20.37	20.53	14.36
152 F	41	17.03	16.02	20.00	.....	14.33
153 NF	53	16.65	15.82	19.48	19.41	14.50
155†NF	....	17.08	15.96	19.15	19.26	12.65

*Main part of the field harvested at this stage.
†Sample taken from the shock.

Table II gives the protein fractions in the flour. The method used in making these determinations is described in detail by Sharp and Herrington (1926). It is probable that the alcohol-soluble fraction is slightly too low and the glutenin fraction slightly too high, because hot alcohol was not used in extracting the gliadin. The method used consisted in the extraction of 4 gm. of flour with 100 cc. of 5% potassium sulfate solution with mechanical shaking for one hour, and then extracting the residue with 70% alcohol for one hour in a mechanical shaker. The protein in the residue after the last extraction, after applying small corrections, was considered as glutenin. The results given in Table II are expressed graphically in the figure. The percentage of

total protein as glutenin in the flour from non-frozen wheat remains practically constant during the period of development studied. At the immature stages of kernel development the frozen samples contain slightly less glutenin than the non-frozen samples. In the early stages, the 5% potassium sulfate soluble protein decreases as the kernel develops and the alcohol soluble protein increases.

TABLE II

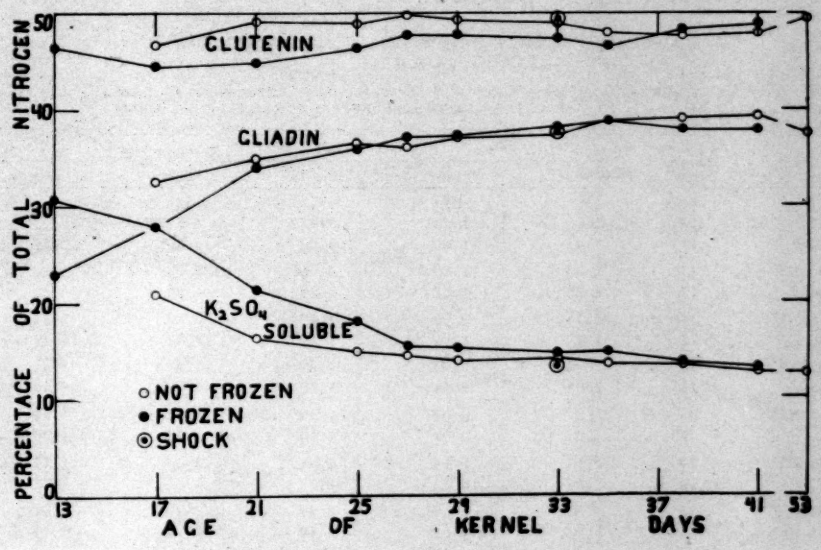
PROTEIN FRACTIONS OF FLOUR MILLED FROM FROZEN (F) AND NON-FROZEN (NF) WHEAT HARVESTED AT VARIOUS STAGES OF MATURITY

Lab. No.	5% K_2SO_4 Percentage of total		Alcohol sol. in residue Percentage of total		Residue glutenin Percentage of total		Total protein sum	Total protein direct
	Flour	Nitrogen	Flour	Nitrogen	Flour	Nitrogen	%	%
131 NF	....	....	....	....	....	....	....	15.07
132 F	4.30	30.7	3.23	23.0	6.48	46.3	14.01	14.60
133 NF	3.01	20.9	4.67	32.5	6.69	46.6	14.37	14.39
134F	3.90	27.9	3.86	27.7	6.20	44.4	13.96	14.47
135 NF	2.32	16.2	5.00	34.8	7.04	49.0	14.36	14.62
136 F	3.16	21.3	5.02	33.9	6.64	44.8	14.82	15.27
137 NF	2.21	14.8	5.46	36.4	7.32	48.8	14.99	15.10
138 F	2.59	18.0	5.14	35.7	6.66	46.3	14.39	14.47
139 NF	2.21	14.3	5.58	36.0	7.70	49.7	15.49	15.62
140 F	2.40	15.4	5.75	37.0	7.41	47.6	15.56	15.47
141 NF	2.25	13.9	5.97	36.9	7.97	49.2	16.19	15.91
142 F	2.59	15.1	6.36	37.2	8.17	47.7	17.12	17.14
145 NF	2.21	14.0	5.87	37.2	7.71	48.8	15.79	16.08
146 F	2.34	14.6	6.12	38.1	7.60	47.3	16.06	16.37
147 NF	2.22	13.4	6.40	38.7	7.93	47.9	16.55	16.39
148 F	2.38	14.8	6.23	38.7	7.48	46.5	16.09	16.07
149 NF	2.20	13.4	6.39	39.0	7.80	47.6	16.39	16.42
150 F	2.25	13.8	6.16	37.9	7.86	48.3	16.27	16.09
151 NF	2.08	12.7	6.41	39.3	7.83	48.0	16.32	16.47
152 F	2.13	13.3	6.04	37.7	7.85	49.0	16.02	16.02
153 NF	2.10	13.2	5.90	37.3	7.83	49.5	15.83	15.82
155 NF	2.10	13.2	5.99	37.5	7.88	49.3	15.97	15.96

The flour from the frozen samples contains a greater percentage of total protein which is soluble in 5% potassium sulfate solution than does the flour from non-frozen wheat. This is especially true in the early stages of development. Freezing apparently affects the gliadin fraction only in the very early stages. The data in the figure are very similar in character to the data in Figure 2 of the paper by Sharp and Elmer (1924) and confirm their conclusions, except that in these experiments there is a tendency for the immature frozen wheat to contain slightly more potassium sulfate soluble protein and slightly less glutenin than the non-frozen wheat harvested at the same stage of maturity. This difference may be due to the fact that the experimental freezing was carried out at much lower temperatures on the samples reported on

in this paper, i. e., —20 to —28° C. as compared with a temperature of —2 to —3° C. used by Sharp and Elmer.

The fact that the frozen samples contain more potassium sulfate soluble nitrogen could be predicted from the data presented in Table VII by Sharp (1925). Here it was shown that if a potassium sulfate solution extract was made of the freshly threshed immature kernels before they had time to dry, an appreciable



Protein Fractions of Flour Milled from Frozen and Non-Frozen Wheat Harvested at Various Stages of Growth

amount of nitrogen was removed; also that if the kernels were allowed to stand in an atmosphere saturated with water vapor, the amount of nitrogen extractable with potassium sulfate decreased; but if the kernels were frozen before this holding in a saturated atmosphere took place, the decrease in nitrogen soluble in potassium sulfate solution was much less. It should be borne in mind when comparing these two sets of data that altho the tables both refer to the same samples, the determinations in the one case were made on whole wheat and in the other case on flour.

Sharp and Elmer (1924) state that Teller (1898) did not determine the protein fractions in the flour milled from wheat harvested at various stages of maturity. This statement was in error. Teller did determine the protein fractions in flour milled from wheat harvested at various stages of maturity and found the per-

centage of total nitrogen in the various protein fractions was very uniform. Thus the results obtained by Teller (1898) and by Sharp and Elmer (1924), and those reported here are all in essential agreement, except that the series of samples used by Sharp and Elmer as well as those reported here included flour milled from more immature wheat than did the series investigated by Teller. At the most immature stages, a decrease in potassium sulfate soluble protein and an increase in alcohol soluble protein were found to be correlated with development.

TABLE III

ASH CONTENT OF WHEAT AND MILL PRODUCTS (DRY BASIS) MILLED FROM FROZEN (F) AND NON-FROZEN (NF) WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY

Lab. No.	Approx. age of kernel	Ash Content				
		Wheat	Flour	Shorts	Bran	Scourings
	Days	%	%	%	%	%
131 NF	13	2.85	1.34	3.47	4.67	....
132 F	13	2.47	1.80	3.00	3.69	....
133 NF	17	2.33	1.00	4.06	5.30	....
134 F	17	2.25	1.62	3.25	4.06	....
135 NF	21	2.14	0.69	3.92	6.13	7.11
136 F	21	2.17	1.34	3.52	4.72	5.47
137 NF	25	1.93	0.65	4.49	7.11	5.42
138 F	25	2.05	1.11	3.30	4.98	5.47
139 NF	27	1.87	0.64	4.30	7.24	4.99
140 F	27	2.03	0.91	4.19	5.72	4.29
141 NF	29	2.11	0.64	4.58	7.33	5.17
142 F	29	2.07	0.84	4.72	6.43	3.98
145*NF	33	1.83	0.60	4.49	7.28	5.07
146 F	33	1.92	0.68	4.49	6.74	3.65
147 NF	35	1.92	0.56	3.72	6.67	4.80
148 F	35	1.93	0.67	4.11	6.69	3.55
149 NF	38	1.90	0.57	4.41	7.07	4.44
150 F	38	1.84	0.63	4.40	6.81	3.78
151 NF	41	1.89	0.52	4.42	6.93	4.29
152 F	41	1.87	0.58	4.19	....	4.31
153 NF	53	1.93	0.57	4.29	7.04	4.20
155†NF	..	1.99	0.62	4.41	6.95	4.26

*Main part of the field harvested at this stage.
†Sample taken from the shock.

Table III gives the ash content of the mill products. The percentage ash content of the whole wheat decreases during development, as has been shown by Kedzie (1893), Teller (1898), and others. Table III indicates a decrease in the percentage ash content of the wheat, flour, and scourings, and an increase in the ash content of the shorts and bran as the kernel develops. The mill products at the earlier stages of kernel development contain more nearly the same percentage of ash than at the later periods. In every case, the flour from the frozen wheat was found to have a

higher ash content than that from the non-frozen sample harvested at the same stage of maturity. The results indicate that the bran and possibly the scourings from the immature frozen samples contain less ash than the bran and scourings from the non-frozen samples harvested at the same stage of development.

The fact that the flour milled from frozen wheat harvested at immature stages of development contains more ash than that from the non-frozen wheat harvested at the same stage of development, can be explained on the basis that freezing tends to change the permeability of the cell membranes, permitting a more nearly equal distribution of the ash throughout the kernel, instead of keeping the ash concentrated in the outer portions of the kernels, as is the case with the non-frozen kernels.

Bailey and Collatz (1921) have shown the relation between the ash content of flour and the electrical conductivity of the aqueous extract. The electrical conductivity and the titratable acidity of the water extracts of this series of flours were determined. Ten-gram samples of flour were introduced into 250 cc. sterilizer bottles. The bottles were kept in a water thermostat for 30 minutes and 100 cc. of water of a temperature of 25° C. was added. The stoppered bottles were then kept in the water thermostat at 25° C. for exactly 30 minutes. During this period the bottles were shaken occasionally. The suspension was centrifuged and 50 cc. of the supernatant liquid was titrated with sodium hydroxide solution, using phenolphthalein as an indicator. Some of the remaining extract was introduced into a conductivity cell and the conductivity determined at 25° C. The conductivity cell was allowed exactly 10 minutes to come to the temperature of the water bath. The results obtained are given in Table IV.

This table, together with Table III, shows the relationship between the ash content of the flour and the conductivity of the water extract, as first demonstrated by Bailey and Collatz; and the relationship between the ash and the titratable acidity as shown by Swanson, Willard, and Fitz (1915). Thus when we compare the flour milled from immature frozen and non-frozen wheat harvested at the same stage of maturity, we observe that the latter contains a lesser amount of ash, and the water extract has a lower titrable acidity and conductivity. It might be mentioned here that the frosted wheat flour also has the higher buffer value, and a slightly lower pH. We observe practically no change in the electrical conductivity and titratable acidity on aging the flour for

10 months at room temperature. The moisture content of the samples averaged 10 to 11 per cent. The conductivity and titratable acidity of the flour milled from the same samples of wheat one year later, as compared with the first milling, showed slight variations which were probably due to the difference in milling, but the differences between the flour from frozen and non-frozen wheat were still as much in evidence as before.

TABLE IV

CONDUCTIVITY AND ACIDITY BY TITRATION OF AQUEOUS EXTRACTS OF FLOUR

The titration figures represent the cc. of N/14 NaOH required to neutralize to phenolphthalein 50 cc. of a solution prepared by extracting 10 grams of flour for 30 minutes with 100 cc. of water at 25°C.

Lab. No.	Conductivity x 1000			Titration		
	First milling Dec. 27, 1923		Second milling Dec. 29, 1924	First milling Dec. 27, 1923		Second milling Dec. 29, 1924
	Mar. 5, '24	Jan. 5, '25	Jan. 5, '25	Mar. 5, '24	Jan. 5, '25	Jan. 5, '25
				cc.	cc.	cc.
131 NF	.....	.....	.....	.....	.....	.....
132 F	.....	1.693	.....	.....	6.90	.....
133 NF	0.815	0.828	.....	2.28	2.40	.....
134 F	1.554	1.562	.....	6.10	6.20	.....
135 NF	0.628	0.633	0.619	1.50	1.55	1.25
136 F	1.324	1.330	1.346	4.50	4.75	4.50
137 NF	0.578	0.573	0.604	1.30	1.35	1.30
138 F	1.082	1.076	1.127	3.45	3.55	3.65
139 NF	0.562	0.557	0.571	1.33	1.30	1.25
140 F	0.831	0.834	0.876	2.45	2.40	2.55
141 NF	0.552	0.541	0.514	1.30	1.28	1.15
142 F	0.752	0.753	0.765	2.28	2.18	2.15
145 NF	0.513	0.511	0.539	1.18	1.15	1.20
146 F	0.602	0.608	0.607	1.58	1.50	1.55
147 NF	0.487	0.494	0.528	1.09	1.05	1.15
148 F	0.617	0.617	0.658	1.49	1.44	1.60
149 NF	0.470	0.473	0.457	1.10	1.00	1.00
150 F	0.525	0.523	0.520	1.25	1.12	1.15
151 NF	0.437	0.445	0.505	1.00	0.98	1.25
152 F	0.462	0.472	0.531	1.17	1.10	1.30
153 NF	0.450	0.460	0.463	1.05	1.08	1.05
155 NF	0.503	0.503	0.487	1.20	1.20	1.08

In Tables VII and VIII of the paper by Whitcomb and Sharp (1926), data are given which were obtained with samples frosted in the field at various stages of maturity. It is difficult to interpret truly the results obtained from wheat frosted in this manner, for no two samples were subjected to the same physiological conditions. The frozen samples of this series were undergoing kernel development in September and October when climatic conditions are quite different from those in August, which is the normal period of kernel development for spring wheat in this region.

TABLE V

PROTEIN IN WHEAT AND MILL PRODUCTS (DRY BASIS)
Frosted in the field

Lab. No.	Damaged wheat	Protein			
		Wheat	Flour	Shorts	Bran
	%	%	%	%	%
1833	0.9	14.57	13.35	18.23	16.01
1834	1.0	15.87	14.96	18.68	17.02
1835	2.3	15.93	13.64	17.72	15.95
1836	71.0	16.13	14.62	20.18	17.98
1837	91.5	15.89	14.69	18.93	17.98
1838	97.4	14.06	12.55	17.49	14.36
1839	100.0	14.55	12.92	17.60	15.21

The percentage of crude protein content of mill products obtained from this series of samples is given in Table V. Little influence of the freezing on the protein content of the mill products is evidenced, unless it is that there is a tendency for the shorts and bran of the two most immature samples to contain less protein than the other samples.

TABLE VI

ASH IN WHEAT AND MILL PRODUCTS (DRY BASIS)
Frosted in the field

Lab. No.	Wheat	Flour	Shorts	Bran
	%	%	%	%
1833	1.82	0.60	4.58	6.56
1834	1.94	0.58	4.53	7.16
1835	1.84	0.65	4.59	6.63
1836	1.71	0.61	4.83	5.95
1837	1.74	0.70	4.08	5.36
1838	1.86	0.68	3.64	4.47
1839	2.17	0.89	3.40	4.47

Table VI indicates a more uniform distribution of the ash in the kernels of the immature frosted wheat, thus confirming, with wheat frosted under field conditions, the results obtained with the experimentally frozen samples, as shown in Table III.

Summary and Conclusions

1. The protein and ash content of wheat and mill products obtained from frozen and non-frozen wheat harvested at various stages of maturity were determined.

2. The amount of ash in flour milled from frozen wheat is slightly higher than in flour milled from non-frozen wheat harvested at the same stage of maturity. This difference in ash content is also indicated by titratable acidity and electrical conductivity.

3. Protein fraction analysis of flour milled from wheat harvested at various stages of maturity indicates no change in the glutenin, an increase in the gliadin, and a decrease in the 5% potassium sulfate soluble protein fractions, as the kernel develops.

4. The flour milled from frozen wheat contains a slightly greater percentage of 5% potassium sulfate soluble protein and a slightly smaller percentage of glutenin than the flour milled from non-frozen wheat at immature stages of development, as shown by the method of protein fraction analysis used in this investigation.

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MAKING LIGHT BREAD FROM MISSOURI SOFT WHEAT FLOUR

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Bread, altho not satisfactory in mineral and vitamin content, can still be called "the staff of life," as there is no other article of diet so universally used. In nearly every part of the world some form of grain product is found to be the largest single source of energy, in the food supply. (Sherman, 1924).

Wheat is commonly divided into two main classes, winter and spring or, in general, soft and hard. Missouri ranks first of all the states in the production of soft winter wheat. Ninety-four per cent of the wheat grown in Missouri is soft, but only 15 per cent of this wheat is used within the state. This is probably due to the claim that soft winter wheat flour is not desirable for making yeast bread.

A great amount of research has been done with flour. Many investigations have been carried on to determine the relation between its chemical composition and its bread making quality. Numerous chemists have long been experimenting to find flour improvers, yeast stimulants, and chemicals to improve flours of poor bread making quality. Little work, however, has been done with soft winter wheat flour to determine, from the housewife's standpoint, the modifications in the proportion of ingredients and in the procedure necessary and possible for her to make good light bread from soft winter wheat flour.

The present investigation of the problem of making light bread from Missouri soft wheat flour was undertaken for two very vital reasons: (1) Alsberg (1924) of the Food Research Institute at Stanford University, contends that there is certainly a hard wheat shortage in store for us, due in part to increase in population, but in a larger measure to increased demand by the bakers for the "stronger" flours. (2) Missouri ranks first in the production of soft winter wheat. The importance of using home-grown products has been emphasized ever since the World War, but even so the majority of the bakers and half of the housewives use flour from other states for making light bread. Missouri people use 17 million bushels of wheat annually, but only 16 per cent of this is produced within the state.

Bread costs more in Missouri than it should because of the double transportation charges. A large part of the wheat is milled out of the state with the result that the supply of mill feed available to Missouri farmers is greatly reduced. Additional feed must be shipped in at a high price and this increases the cost of milk and meat. When we increase the cost of bread, milk, and meat we have increased the cost of three of our most important articles of diet.

The first portion of this study has been confined to experimental work on making good light bread by the use of the straight dough method and compressed yeast, with one brand of soft winter wheat flour. The second division of the work was with dried yeast.

The flour used for the experimental work was Boone County high patent flour, made from soft red winter wheat grown in Central Missouri. Tap water was used as the liquid and was weighed at 35° C. Lard was selected as the shortening. The ingredients for each loaf of each experiment were weighed and mixed separately. The proportions were for one-pound loaves.

Number of Risings in Fermentation Period

There are different opinions, even among the few who have worked with soft wheat flour, as to the number of kneadings or risings which give the best results. Therefore, the first series of experiments was to determine the proper length of the fermentation period.

TABLE I
NUMBER OF RISINGS IN FERMENTATION PERIOD

No. of loaves	No. of risings in cyl.	Amt. of rising in cyl.	Amt. of proofing in pan	Loaf vol.	Total time	
				cc.	hr.	min.
4	0	none	treble	1680	2	40
8	1	treble	treble	1725	3	45
15	2	treble	treble	1720	4	30
4	3	treble	treble	1740	5	00
8	2	treble treble	treble	1710	4	30
6	2	treble treble	double	1560	4	20
4	2	treble treble	one ¾	1500	4	00
4	2	double double	double	1500	3	50
4	2	double treble	double	1520	4	30
6	2	double double	treble	1700	4	00
4	1	treble	one ¾	1510	3	00
4	1	treble	double	1580	3	30
6	1	treble	two ¾	1716	3	30
3	1	four X bulk	treble	1600	4	00
4	1	double	double	1640	2	40
8	1	double	treble	1740	3	00

TABLE II
COMPARISON OF BEST PROCEDURES FROM TABLE I

No. of loaves	No. of risings in cyl.	Amt. of rising in cyl.		Amt. of proofing in pan	Loaf vol.	Total time	
		1st	2d		cc.	hr.	min
8	2	treble	treble	treble	1710	4	30
6	2	double	double	treble	1700	4	00
8	1	treble	.....	treble	1710	3	45
6	1	treble	.....	two ¾	1700	3	30
8	1	double	.....	treble	1750	3	00

These experiments show that soft winter wheat flour gives best results when the time of fermentation is short, but when the proofing is prolonged until the dough has trebled its volume in the pan. At this volume the dough gives a little resistance when touched lightly with the finger. This amount of proofing overcomes the close, cake-like, texture and small volume, which characteristics have led bakers to discriminate against the use of this type of flour in bread making. Longer proofing preceded by a short fermentation period gives bread of open texture and good volume. The best results were obtained by allowing the dough to double its volume in the cylinder and to treble its volume in the pan. This method of procedure was followed for all the experiments.

The object of the following series of experiments was (1) to determine the effect of the proportion of ingredients on the loaf volum and texture of the bread; and (2) to determine the proportions which give the best results with the flour and procedure used.

TABLE III
VARIATION IN PROPORTION OF LIQUID

No. of loaves	Water	Wt. of loaf 30 min. after baking	Loaf volume
	%	gm.	cc.
4	48	440	1550
10	49	443	1580
4	50	458	1630
12	51	444	1700
16	52	453	1730
16	53	455	1730
4	54	453	1715
4	55	459	1700
4	56	460	1610

To a certain point, loaf volume and loaf weight increase with an increase in the proportion of liquid used. The texture is close, cake-like, and uneven with a small percentage of water (a stiff dough); becomes more open and uniform in texture, within certain limits, with an increase in percentage of water; and becomes quite coarse and crumbly, with too much water.

In this series of experiments the best results were obtained when the dough was just stiff enough to hold its shape and to develop a bold, nicely rounded, top surface when proofed, but so soft that it required quick handling to prevent its adhering to the board and hands during kneading and moulding. This consistency was obtained by the use of 52% water.

TABLE IV
VARIATION IN PROPORTION OF YEAST

No. of loaves	Yeast	Wt. of loaf 30 min. after baking	Loaf vol.	Total time	
	gm.	gm.	cc.	hr.	min.
4	3.5	441	1430	4	30
4	7.0	445	1600	3	40
14	10.0	456	1710	3	00
14	14.0	457	1760	2	22
10	21.0	462	1790	2	30
6	28.0	462	1740	2	10
4	42.0	464	1700	3	20
4	56.0	468	1670	2	40

This series shows that with increased yeast there is a corresponding increase in volume up to 28 gm. or two cakes per loaf, an increase in weight, and a decrease in total time for rising. With increased yeast, the texture becomes more coarse, tender, and spongy, until finally it is very crumbly. Yeast beyond two cakes per loaf makes the gluten so soft that it loses its capacity for holding gas and thus the cell walls collapse and a poor oven spring results.

TABLE V
VARIATION IN PROPORTION OF SUGAR

No. of loaves	Sugar	Wt. of loaf 30 min. after baking	Loaf vol.	Total time	
	gm.	gm.	cc.	hr.	min.
4	4.3	438.5	1500	3	30
4	8.6	445	1610	3	20
18	10.0	450	1710	2	20
18	17.2	455	1820	2	30
4	25.8	479	1870	2	00
4	34.4	486	1900	1	50
4	38.7	490	1900	1	40

These experiments show that with an increased amount of sugar there is a corresponding increase in loaf volume, weight of loaf, and quality of texture. The oven spring is excellent when large quantities of sugar are used; the crust browns very quickly and becomes very deep in color and thick around all sides of the loaf. The texture becomes especially spongy, silky, and moist. Increased sugar seems to have a toughening action on the gluten.

Sugar is necessary for the production of good "bloom" or color of loaf, especially when the bread is made from soft flour. Sugar also helps to retain freshness, because the loaf takes on the desired color readily, allowing less water evaporation. Seventeen grams of sugar gave the best results. More than this amount gave too sweet a taste to the bread.

TABLE VI

VARIATION IN PROPORTION OF SALT

No. of loaves	Salt	Wt. of loaf 30 min. after baking	Loaf vol.	Total time	
	gm.	gm.	cc.	hr.	min.
22	5	472	1870	2	25
12	6	475	1755	2	45
22	7	476	1737	2	55
4	8	478	1730	3	20
4	9	478	1776	3	30
4	11	487	1670	3	49
4	13	489	1635	3	59

Salt in small amounts makes the crumb of bread whiter, the dough easier to handle because the gluten is thus more elastic, retards fermentation, improves the color of the crust and brings out the nutty flavor of the baked wheat grain. Salt increased beyond 5 gm., or about one teaspoon, increases the weight of the loaf and the length of time required for rising and decreases the volume. With larger amounts of salt there is, further, a noticeable loss in color of the crust, flavor, and tenderness and fineness of texture. The texture becomes coarse, tough, rubbery, and dark in color.

TABLE VII

VARIATION IN PROPORTION OF SHORTENING

No. of loaves	Fat	Wt. of loaf 30 min. after baking	Loaf vol.	Total time	
	gm.	gm.	cc.	hr.	min.
4	3.3	471	1795	2	50
22	6.5	472	1870	2	25
22	9.8	475	1895	2	40
6	13.0	477	1910	2	49
6	16.3	481	1915	2	46
6	19.5	482	1830	2	43
4	26.0	487	1760	2	31

An increase in the proportion of shortening results in increased weight, increased volume (to certain limits), elasticity and softness of gluten; also in decreased length of rising period and loaf volume, after two teaspoons per loaf. A little shortening gives a finer and more silky texture of crumb and a sheen and velvetyness of pile, as in the case of bread from hard wheat flour.

Shortening also helps to prevent drying and adds nourishment. With the larger amount of lard, the texture becomes more tender, crumbly, heavy, and yellow in color; the crust does not attain a rich brown color.

These experiments show that the small amount of gluten in the type of flour used requires a short fermentation period but a proofing period long enough to treble its volume. It requires gentle kneading and mixing, increased yeast, increased sugar, and a soft or slack dough to bring about the desired loaf volume and the moist, spongy, and open texture associated with good bread. With the proper procedure and proportion of ingredients, good bread can be made from soft winter wheat flour in a very short time.

The second division of the experimental work dealt with the use of dried yeast with Missouri soft wheat flour. The formula and method worked out above call for a large quantity of compressed yeast. This is not practical for the farm woman or the housewife in the small town. Compressed yeast must be perfectly fresh to do its best work, and it is also the most expensive kind of yeast. It is often advantageous to use a cheaper yeast which can be kept in good condition for a long time.

The experimental work has been carried out along the following lines:

1. A study of the effect of procedure on the quality of the bread, with reference to the length of the preliminary fermentation period.

2. A study of the effect of the addition of various ingredients to the prelimiary fermentation mixture and to the dough, on loaf volume and the quality of the bread.

The problem was begun by determining the comparative gas production of dried and compressed yeast (by means of fermentation tubes). Results showed that it took 2 hours and 10 minutes for the same amount of dried yeast to produce as much gas as the compressed yeast did in 30 minutes. Several fermentation tests were run to find variations of gas-producing power in one cake and in one package of dried yeast. These showed that the amount of gas produced by one portion of a cake of dried yeast varied greatly from that produced by another portion of the same cake, giving a coefficient of variation of 43 per cent. Experiments using fermentation periods varying from 2 hours and 10 minutes to 6 hours and 35 minutes showed that a large quantity of dried yeast would not

produce good results, even tho the fermentation period was short. The crumb was very dark and the loaf was of poor shape and low volume, owing to the large quantity of cornmeal in the yeast. As a large quantity of dried yeast would never give a crumb of good color, the amount was cut down with variation in the length of the preliminary fermentation period.

TABLE VIII

VARIATION IN LENGTH OF PRELIMINARY FERMENTATION PERIOD

No. of loaves	Pre. fer. period	Loaf vol.	Total time after pre. fer. period	
	hr.	cc.	hr.	min.
5	12	1556	7	58
6	18	1600	7	18
3	24	1543	7	42
3	36	1500	8	30

None of the experiments of this series gave satisfactory bread from the standpoint of either texture or loaf volume, which seemed to show that the small amount of dried yeast needed something more in the long preliminary fermentation period than just water and sugar. It was found that by slightly increasing the sugar and adding flour in the preliminary fermentation period the quality of bread could be slightly improved.

In all previous experiments the fermentation and proofing periods were very long as compared with those when compressed yeast was used. Also the shorter fermentation and proofing periods gave the best results with dried yeast.

A study of the chemical composition of soft and hard wheat flours shows that soft wheat flours contain less minerals than hard wheat flours. It is contended that one of the factors of strength of flours depends on the relation between the concentration of the acids and soluble salts in the flour (Blish, 1916), (Martin, 1920), and that minerals seem to strengthen the gluten and hasten fermentation (Harcourt and Purdy, 1922), therefore potato water was used as the liquid. Results showed a greatly improved product but not a perfect one. Adding gelatinized starch in the form of potato or scalded flour gave excellent results.

Buttermilk contains 8.5% milk solids and a little fat. It has a softening effect on gluten because of its acidity. Doughs made with buttermilk mature much faster than those made with water, thus the fermentation period is shortened (Gerhard, 1925).

Buttermilk used as the liquid gave a loaf of greater volume, better texture, more oven spring, and a much shorter total time than bread made with milk.

By adding lactic acid to milk, bringing it to the same acidity as the buttermilk, comparable results were obtained.

TABLE IX

EFFECT OF ADDITION OF MINERALS AND ACIDS

No. of loaves	Pre. fer. period	Kind of liquid used	Kind of gelatinized starch used	Loaf vol.	Wt. of loaf 30 min. after baking	Total time after pre. fer. period	
	hr.			cc.	gm.	hr.	min.
9	18	plain water	none	1570	455	5	21
3	12	plain water	none	1447	450	6	5
21	18	potato water	16.2 gm. scalded flour	1605	460	4	10
9	12	potato water	75 gm. potato	1825	482	2	42
6	12	potato water	25 gm. potato	1810	452	2	55
13	12	potato water	16.2 gm. scalded flour	1773	475	2	45
9	12	⅓ milk ⅔ potato water	75 gm. potato	1665	495	2	49
10	12	⅓ milk ⅔ potato water	16.2 gm. scalded flour	1650	492	3	0
9	12	⅓ buttermilk ⅔ potato water	75 gm. potato	1699	487	2	41
10	12	⅓ buttermilk ⅔ potato water	16.2 gm. scalded flour	1830	483	2	39
7	12	⅓ milk ⅔ potato water 1.5 cc. lactic acid	16.2 gm. scalded flour	1765	490	2	46

These experiments show that potato and scalded flour, as forms of gelatinized starch, gave comparable results.

A slightly larger volume was obtained by the addition of acid in the form of buttermilk.

All loaves had good volume, excellent oven spring, and a fine silky texture with thin cell walls.

Apparatus for Determining Loaf Volumes

The loaf volume was measured by displacement of millet seed, the apparatus being so constructed that the exact volume of the loaf could be read directly. This piece of apparatus consisted of the following parts: A rectangular hopper of sufficient size to contain the loaf to be tested; an auxiliary filling funnel; a measuring burette with a graduated glass front, and a stand provided with two ring clamps for holding the hopper and funnel in place.

Summary

The results of the experiments described have conclusively shown that light bread can be made from the softer flours, using either compressed or dried yeast, comparable in quality to that made from our best hard wheat flours, and in much less time—2½ to 3 hours being the total time after the preliminary fermentation period.

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A RAPID MOISTURE TESTING OVEN FOR CEREAL CHEMISTRY LABORATORIES

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Industrial enterprises are constantly in search of methods of analysis which will so shorten their routine laboratory work as to give more efficient plant control. This is particularly true of the cereal chemist, who has been looking for an apparatus which would allow him to determine the moisture content of flour and mill products in a much shorter time than that consumed by the use of the various air ovens now in use.

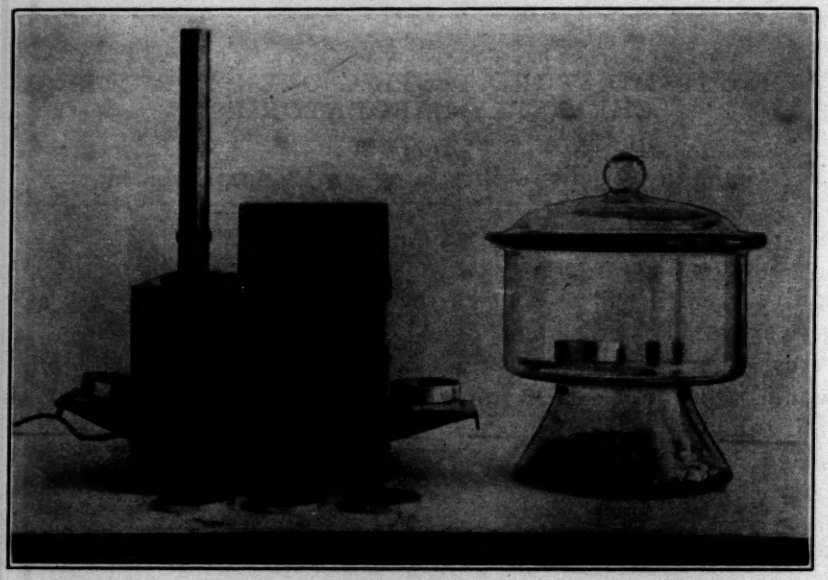
A foreign built air oven has recently arrived which is designed to determine the moisture content of flour, etc., in approximately fifteen minutes. Through the courtesy of a well known firm, one of these ovens was obtained in order to compare its efficiency with that of ovens of accepted efficiency.

This oven, which is shown in the figure, is quite different in design from the air ovens in use in this country. It is quite small, being 8½ inches long, 7½ inches wide, and 7½ inches high on the outside.

The heating chamber, which is 2 inches high and 3 inches wide, passes lengthwise through the oven. It is provided with closely fitting asbestos insulated hinged doors on each end of the oven. These doors operate simultaneously and provide for the insertion and removal of the moisture boxes. Three moisture testing boxes can be placed in the heating chamber at one time.

The moisture boxes are of aluminum and are 2½ inches in diameter and ¾ of an inch in height. The boxes are also provided with closely fitting tops.

The oven is heated by electricity. Temperature is controlled by means of a thermo-regulator which is suspended through the top of the oven into the heating chamber. The thermo-regulator consists of an expandable metallic capsule. In the center of this capsule is a small brass rod, which moves upward and downward as the capsule expands and contracts, and operates a lever against the action of a spring to a relay situated on one corner of the top of the oven. The relay throws on and cuts off the current as the temperature requirements within the heating chamber are met.



Oven with Doors Open Showing How Moisture Boxes Are Inserted and Removed. The lever on the side opens and closes both doors automatically. Desiccator to the right.

Ventilation is obtained by means of two series of holes ¼ inch in diameter, and a metal stack 9 inches high and 1 inch in diameter. Air enters the oven through a series of holes located on the outside about ½ inch from the sides of the door at the stack end of the heating chamber, passing over the top and sides of the outside of the heating chamber. The second series of holes is located at the opposite end of the oven within the heating chamber itself. At this point the outside air, which has been previously heated, enters the heating chamber. Further movement of the air is accomplished by means of the 9-inch stack previously mentioned, which is locat-

ed on that end of the oven at which the outside air enters. The air thus circulates in a clockwise direction as it enters and leaves the moisture oven.

The instructions of the manufacturers for operating the oven are given below:

"(1) The current should be switched on at least 30 minutes before it is desired to use the oven, three blank tins inserted, and the doors closed. If the oven is new, or has not been used for some time, it will be necessary to adjust the thermostat by means of the brass milled head under the movable cover on top of the oven. This can be done conveniently without removing the cover by passing a screwdriver through the hole above the brass milled head which is slotted. The thermostat should be so set that the temperature keeps within one or two degrees of 135° centigrade. If the temperature is allowed to exceed 145° the capsule may be damaged, and care should be taken that this does not happen.

"(2) Weigh out **five-grams** of the substance to be tested (to the nearest milligram) and spread evenly over the bottom of the weighing tin. Wheat and coarse grained products such as bran, should be kibbled (ground) as finely as possible before weighing.

"(3) Open the doors of the oven, insert the tin (at the stack end) and remove the tin ejected at the other end and close the doors. This operation should be done smartly to avoid loss of heat.

"(4) Weigh out another sample, and insert it **exactly five minutes** after the first, and continue to do this as long as there are samples to be tested. If, for any reason, the sample is not ready at the proper time, insert an empty tin.

"(5) The first sample to be tested will be ejected at the end of fifteen minutes. This should at once have its lid placed upon it. (Lids are best kept outside the oven, as, being cool, they make it possible to handle the hot tins). Successive samples should be ejected at five minute intervals.

"(6) When all the samples to be tested have been placed in the oven, continue to add blank tins until the last sample has been ejected, then switch off.

"The interval has been set at five minutes to give sufficient time to weigh out one sample for the oven, remove, cool and weigh a dried sample and enter figures in notebook. To do this properly requires smart and intelligent handling on the part of the operator, and in some cases it will be found more convenient to place the dried samples in a desiccator when removed from the oven and to weigh up at leisure later on.

"Whenever possible use three samples of each product to be tested. This reduces the error of sampling, which is usually greater than the error of the oven, and gives an accuracy greater than that given by four hours drying in an old type electric oven. Three samples can be done comfortably in 30 minutes."

The effectiveness of this method was compared with two commonly used methods for making moisture tests on flour and mill products, namely, heating the material for 5 hours at a temperature of 108° C. and heating for 1 hour at a temperature of 130° C. The latter method is the one tentatively adopted by the A. O. A. C. as a substitute for the vacuum-oven test.

Twenty-five samples of wheat, flour, bran, and shorts were tested by all three methods. Unless otherwise stated, the moisture content of 5 grams of each material was determined.

The results of these tests are given in Tables I to IV.

TABLE I
COMPARISON OF THREE METHODS FOR MAKING MOISTURE TESTS ON FLOUR

Sample No.	Moisture content of flour			Difference from A.O.A.C. method	
	5 hr. at 108°C.	1 hr. in 130°C. oven	15 min. at 135°C.	15-minute method	108°C. method
	%	%	%	%	%
1	12.51	12.75	12.69	−.06	−.24
2	12.29	12.56	12.51	−.05	−.27
3	13.15	13.36	13.38	+.02	−.21
4	12.68	12.79	12.73	−.06	−.11
5	12.62	12.85	12.84	−.01	−.23
6	12.76	12.97	12.84	−.13	−.21
7	12.04	12.30	12.24	−.06	−.26
8	12.50	12.75	12.66	−.09	−.25
9	12.43	12.69	12.68	−.01	−.26
10	12.12	12.34	12.20	−.14	−.22
11	12.06	12.34	12.30	−.04	−.28
12	12.48	12.82	12.78	−.04	−.34
13	11.83	12.06	12.04	−.02	−.23
14	12.46	12.61	12.66	+.05	−.15
15	12.24	12.46	12.42	−.04	−.22
16	11.97	12.18	12.16	−.02	−.21
17	11.82	12.26	12.27	+.01	−.44
18	12.44	12.69	12.69	.00	− 25
19	12.18	12.44	12.43	−.01	−.26
20	12.26	12.52	12.49	−.03	−.26
21	13.58	13.86	13.84	−.02	−.28
22	11.78	11.98	12.00	+.02	−.20
23	13.18	13.38	13.32	−.06	−.20
24	9.12	9.36	9.30	−.06	−.24
25	8.78	9.04	9.00	−.04	−.26
Average difference				.044	−.243
Minimum difference				.000	−.11
Maximum difference				−.14	−.44
Maximum spread				±.19	−.44

Table I records the data obtained after a study was made of the moisture content of 25 samples of straight grade flour, the moisture content being determined by the three methods men-

tioned. There was a remarkably close agreement between the results obtained by the A. O. A. C. method and the 15-minute method, the average difference between the two being only 0.04%. The maximum difference in moisture with any one sample was only 0.14%, whereas the entire spread in results in either direction was only 0.19%.

In passing, it is interesting to note that moisture tests accomplished by heating flour for 5 hours, at a temperature of 108° C. gave, on the average, results lower by 0.24% when compared to the A. O. A. C. method, and by 0.20% when compared to the 15-minute method.

Moisture tests were next made on shorts. In Table II a similar close agreement of results can be seen when the data obtained by the A. O. A. C. method and the 15-minute method are compared. The average difference between the two methods in this case was 0.06%. The maximum spread in either direction was only 0.18%. The 5-hour, 108° C. method gave results 0.3% lower than the A. O. A. C. method.

TABLE II

COMPARISON OF THREE METHODS FOR MAKING MOISTURE TESTS ON SHORTS

Sample No.	Moisture content of shorts			Difference from A.O.A.C. method	
	5 hr. at 108°C.	1 hr. in 130°C. oven	15 min. at 135°C.	15-minute method	108°C. method
	%	%	%	%	%
1	10.57	10.82	10.76	−.06	−.25
2	10.38	10.58	10.56	−.02	−.20
3	9.98	10.23	10.17	−.06	−.25
4	9.52	9.78	9.70	−.08	−.26
5	9.82	10.02	10.01	−.01	−.20
6	10.98	11.18	11.14	−.04	−.20
7	11.08	11.42	11.30	−.12	−.34
8	9.96	10.26	10.18	−.08	−.30
9	9.84	10.02	10.06	+.04	−.18
10	11.10	11.50	11.38	−.12	−.40
11	11.36	11.66	11.58	−.08	−.30
12	10.89	11.22	11.12	−.10	−.33
13	11.52	11.96	11.82	−.14	−.44
14	10.50	10.78	10.82	+.04	−.28
15	10.46	10.84	10.82	−.02	−.38
16	11.10	11.49	11.43	−.06	−.39
17	9.84	10.17	10.18	+.01	−.33
18	9.86	10.21	10.25	+.04	−.35
19	11.36	11.66	11.61	−.05	−.30
20	10.44	10.86	10.79	−.07	−.42
21	11.04	11.34	11.28	−.06	−.30
22	10.74	11.00	11.00	.00	−.26
23	11.42	11.68	11.64	−.04	−.26
24	10.80	11.08	11.02	−.06	−.28
25	10.42	10.73	10.62	−.11	−.31
Average difference				.06	−.30
Minimum difference				.00	−.18
Maximum difference				−.14	−.44
Maximum spread				±.18	−.44

With bran, in the same way as before, close agreements were obtained between the A. O. A. C. method and the 15-minute method. These data are given in Table III. The average difference between the two methods mentioned above was again 0.06%, with a maximum difference with any one sample of only 0.16%. The maximum spread in either direction was 0.27%. The results by the 108° C. 5-hour method averaged 0.27% lower than the A. O. A. C. method.

TABLE III

COMPARISON OF THREE METHODS FOR MAKING MOISTURE TESTS ON BRAN SAMPLES

Sample No.	Moisture content of bran			Difference from A.O.A.C. method	
	5 hr. at 108°C.	1 hr. in 130°C. oven	15 min. at 135°C.	15-minute method	108°C. method
	%	%	%	%	%
1	11.31	11.59	11.55	−.04	−.28
2	12.44	12.62	12.78	+.16	−.18
3	12.53	12.84	12.94	+.10	−.31
4	12.56	12.90	12.88	−.02	−.34
5	11.45	11.69	11.68	−.01	−.24
6	11.31	11.67	11.72	+.05	−.36
7	12.74	12.94	12.98	+.04	−.20
8	12.62	12.88	12.90	+.02	−.26
9	11.80	12.09	12.09	.00	−.29
10	12.61	12.97	12.86	−.11	−.36
11	13.16	13.42	13.56	+.14	−.26
12	13.06	13.24	13.29	+.05	−.18
13	13.46	13.73	13.77	+.04	−.27
14	12.39	12.66	12.70	+.04	−.27
15	11.69	11.94	11.94	.00	−.25
16	12.18	12.56	12.70	+.14	−.38
17	13.50	13.77	13.86	+.09	−.27
18	12.15	12.50	12.44	−.06	−.35
19	12.95	13.38	13.44	+.06	−.43
20	12.77	13.04	13.01	−.03	−.27
21	13.14	13.40	13.36	−.04	−.26
22	13.18	13.32	13.34	+.02	−.14
23	14.14	14.40	14.34	−.06	−.26
24	13.98	14.20	14.22	+.02	−.22
25	12.46	12.65	12.60	−.05	−.19
Average difference				.056	−.273
Minimum difference				.00	−.14
Maximum difference				+.16	−.43
Maximum spread				±.27	−.43

The data obtained on ground wheat samples are given in Table IV. While the results with this commodity were not quite so close as when the A. O. A. C. method was compared with the 15-minute method, they yet show a very close agreement, as the average difference was but 0.09%.

It was later found that by raising the temperature to 140° C. for the same interval of time, the average difference between the two methods above discussed was only 0.04%, with a maximum difference with any one sample of only 0.08%. The maximum spread in moisture results in either direction was 0.14%.

TABLE IV

COMPARISON OF THREE METHODS FOR MAKING MOISTURE TESTS ON WHEAT SAMPLES

Sample No.	Moisture content of wheat			Difference from A.O.A.C. method	
	5 hr. at 108°C.	1 hr. in 130°C. oven	15 min. at 135°C.	15 minute method	108°C. method
	%	%	%	%	%
1	10.26	10.52	10.39	−.13	−.26
2	10.14	10.33	10.31	−.02	−.19
3	10.46	10.78	10.63	−.15	−.32
4	10.03	10.27	10.22	−.05	−.24
5	10.18	10.44	10.26	−.18	−.26
6	10.07	10.31	10.14	−.17	−.24
7	10.65	10.89	10.84	−.05	−.24
8	10.48	10.83	10.68	−.15	−.35
9	10.69	10.96	10.88	−.08	−.27
10	9.98	10.16	10.10	−.06	−.18
11	10.76	11.02	10.88	−.14	−.26
12	8.74	9.04	8.96	−.08	−.30
13	8.82	9.06	8.99	−.07	−.24
14	10.08	10.49	10.54	+.05	−.41
15	10.42	10.87	10.73	−.14	−.45
16	11.30	11.71	11.54	−.17	−.41
17	10.48	10.88	10.82	−.06	−.40
18	9.71	10.08	9.95	−.13	−.37
19	11.48	11.92	11.90	−.02	−.44
20	13.01	13.45	13.46	+.01	−.44
21	8.66	9.07	9.02	−.05	−.41
22	9.18	9.62	9.60	−.02	−.44
23	9.72	10.10	9.96	−.14	−.38
24	10.54	10.93	10.93	.00	−.39
25	8.86	9.22	93.2	+.10	−.36
Average difference				.089	−.33
Minimum difference				.00	−.18
Maximum difference				−.18	−.45
Maximum spread				±.28	−.45

Observations Relative to the Operation of the Oven and the Technic of the Test

Before proceeding far with the use of the oven it became apparent that the thermo-regulator was not so sensitive as it should be, as much of the technic of keeping the temperature constant during the period of the test had to be accomplished by hand manipulation. Whether the thermo-regulator used on this type of oven is inherently faulty or whether the fault is only peculiar to the individual oven tested cannot be said. If this difficulty is overcome, the oven is ideal for the purpose intended.

The oven will reach the desired temperature of 135° C. in 30 minutes, as advertised, hence it is not necessary to maintain current flow for 24 hours a day as with larger ovens.

Contrary to the larger ovens, the drop in temperature upon inserting the moisture boxes is never more than 2 degrees when the oven is correctly operated. Moreover, this loss in temperature is regained rapidly. This factor is, of course, time saving.

This slight drop in temperature has no effect on the moisture results, as duplicate determinations checked very well. This is further brought out because seldom, if ever, were duplicate tests in the oven at the same time.

Twelve tests can be completed in an hour and a quarter.

In conclusion, it is felt that if the oven can be supplied with a more sensitive thermo-regulator the method of testing moisture in cereals made possible by its manufacture is an ideal substitute for the 130° C. air-oven method recommended by the A. O. A. C.

A RAPID ELECTROMETRIC METHOD FOR THE MEASUREMENT OF HYDRION CONCENTRATION OF FLOUR-WATER SUSPENSIONS

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A Correction

The pH conversion curve of the quinhydrone-saturated calomel cell presented as Figure 2 of our paper in the May, 1926, issue was incorrectly drawn. A corrected curve appears below which should replace the curve appearing on page 161 of Volume III.

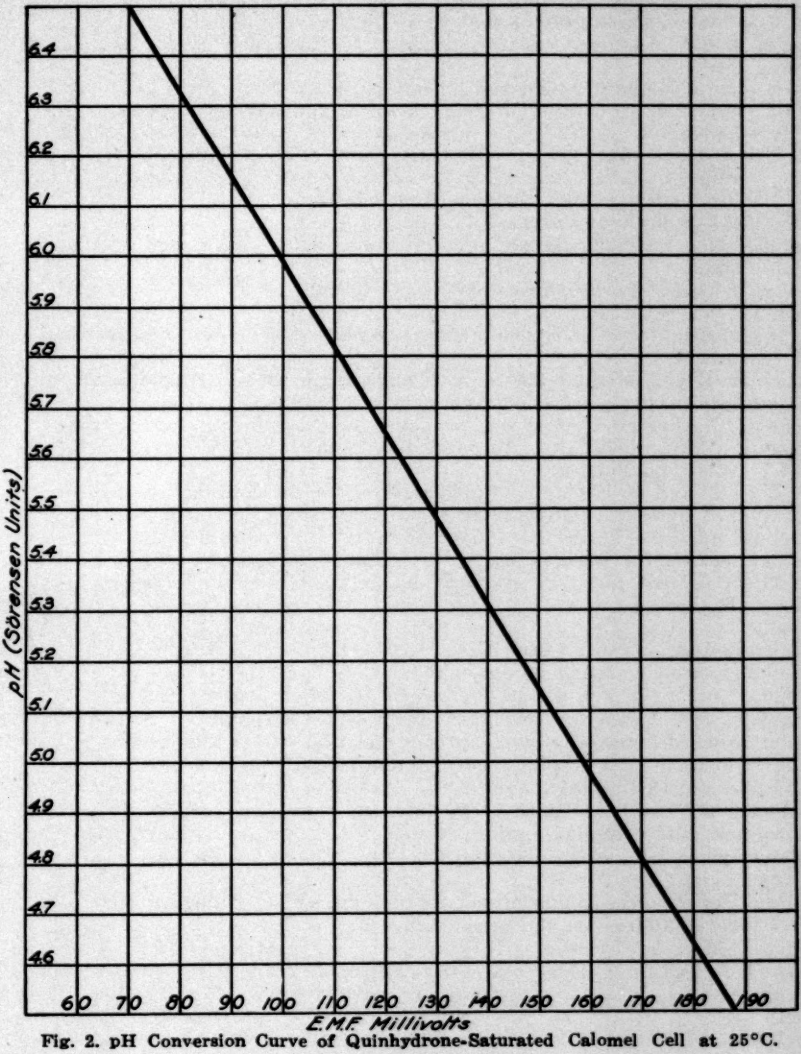

Fig. 2. pH Conversion Curve of Quinhydrone-Saturated Calomel Cell at 25°C.

INDEX—AUTHOR AND SUBJECT

Page

Absorption, effect on properties of bread. C. G. Harrel..... 2
Acid, standardization for protein determinations. D. A. Coleman, et al..... 255
Acidity, relation to bread production. C. G. Harrel 1
Amos, Percy A. "Processes of flour manufacture." Review by C. H. Bailey 199
Ash determination
  Collaborative study of methods. D. A. Coleman, et al..... 267
  Comparison of official method with Hertwig and Bailey method. C. F. Rogers 226
  Oxygen-acetate method. G. L. Brendel..... 222
Baart, hard, a superior wheat variety for western agriculture. C. H. Briggs 343
Bailey, C. H. Review of "Processes of flour manufacture" by P. A. Amos.. 199
Bailey, C. H. and R. C. Sherwood. Control of diastatic activity in wheat flour. I. Production of diastatic flour and effect of large dosages on wheat flour 107
  II. Experiments with flour milled on a commercial scale..... 165
  Relation of crude protein content of flour to loaf volume..... 393
Baking quality, relation to protein content. C. E. Mangels..... 150
Baking test
  Report of committee on standardization. L. A. Fitz..... 252
  A discussion of certain of their chemical and physical aspects. F. L. Dunlap 201
  For biscuits baked from self-rising flour. Paul Logue and Irene T. Ranker 336
  The problem of standardizing. M. J. Blish..... 216
Ball, C. D. A study of wheat oil..... 19
Biscuits, and leavening agents for self-rising flour. P. Logue and I. T. Ranker 337
Blackhull wheat, characteristics of. R. S. Herman..... 244
Blair, G. W., Scott and H. J. Denham. A rapid electrometric method for the measurement of hydrion concentration of flour-water suspensions 158
  Correction 428
Bleaching flour, should flour be artificially matured and decolorized. M. Javillier (trans. by Alma E. Warthen) 359
Blish, M. J. The problem of standardizing the experimental baking test.... 216
  and R. M. Sandstedt. An improved method for the preparation of wheat gliadin 144
Bohn, R. T. and R. J. Martz. Rapid method for the colorimetric determination of hydrogen-ion concentration of crackers..... 183
Book reviews
  "Handbook for Bakers," by A. F. Gerhard. Reviewed by C. G. Ferrari 63
  "Processes of flour manufacture" by Percy A. Amos. Reviewed by C. H. Bailey 199
Bread
  From Missouri soft wheat flour. E. M. Davis and J. A. Cline..... 411
  Some variable factors of production. C. G. Harrel 1
Brendel, G. L. Oxygen-acetate method of ash determination in flour..... 222
Briggs, C. H. A superior new wheat for western agriculture..... 343
Buffer value of flour, relation to bread production. C. G. Harrel..... 11
Cakes, a method for a graphic record of texture, volume, and contour. A. M. Child and D. I. Purdy..... 57
Child A. M. and D. I. Purdy. Method for a graphic record of texture, volume, and contour of cakes..... 57
Calcium phosphate, effect upon the viscosity of flour suspensions. L. Earlenbaugh 102
Chlorine, effect upon baking properties of flour. F. L. Dunlap..... 201
Clark, R. J. Address of the president..... 282
Climate, effect on diastatic activity of flour. C. E. Mangels 318
Cline, J. A. and E. M. Davis. Making light bread from Missouri soft wheat flour 411

Christie, Alfred, and D. A. Coleman. A rapid method for determining the gasoline color value of flour and wheat 84
The gasoline color value of several classes of wheat 188
Coleman, D. A.
Report of committee on methods of analysis 254
and Alfred Christie. A rapid method for determining the gasoline color value of flour and wheat 84
The gasoline color value of several classes of wheat 188
and H. B. Dixon. A rapid moisture testing oven for cereal chemistry laboratories 419
Color
Rapid method for determining gasoline color value. D. A. Coleman and A. Christie 84
Gasoline color value of several classes of wheat. D. A. Coleman and A. Christie 188
Convention minutes, 1926. R. K. Durham 289
Crackers, rapid colorimetric determination of hydrogen-ion concentration. R. T. Bohn and R. J. Martz 183
Crude protein of flour, relation to loaf volume. C. H. Bailey and R. C. Sherwood 393
Davidson, J. and J. H. Shollenberger. Effect of sodium nitrate applied at different stages of growth of wheat on the baking quality of the flour.. 137
Davis, E. M. and J. A. Cline. Making light bread from Missouri soft wheat flour 411
Denham, H. J. and G. W. Scott Blair. A rapid electrometric method for measurement of hydrion concentration of flour-water suspensions.... 158
Correction 427
Diastatic activity
Production of diastatic flour and effect of large dosages. R. C. Sherwood and C. H. Bailey 107
Experiments with flour milled on a commercial scale. R. C. Sherwood and C. H. Bailey 163
Factors affecting. C. E. Mangels 316
Of wheat flour. C. E. Mangels 154
Diastase, and certain reactions of starch. A. J. Hermano and O. S. Rask.. 361
Dixon, H. B. and D. A. Coleman. A rapid moisture testing oven for cereal chemistry laboratories 419
Dough
Effect of freezing. W. O. Whitcomb and P. F. Sharp 303
Mechanical modification of. C. O. Swanson and E. B. Working 65
Dunlap, F. L. The problem of test bakes with a discussion of certain of their chemical and physical aspects 201
Dunn, J. A. Plasticity—its possibilities in cereal research 351
Durham, R. K. Minutes of the twelfth convention, 1926 289
Secretary-Treasurer's report 291
Durum wheats, relation of protein content to baking quality. C. E. Mangels 150
Earlenbaugh, L. Effect of mono-calcium phosphate upon the viscosity of flour-in-water suspensions. 103
Embryo, a study of the oil. C. D. Ball 19
Epstein, A. K. and B. R. Harris. Detection of minute amounts of naphthalene in flour 60
Fat, a study of wheat. C. D. Ball 19
Fermentation
Effect of yeast on flour proteins. P. F. Sharp and O. M. Schreiner.... 90
Effect on properties of bread. C. G. Harrel 6
and mechanical modification of dough. C. O. Swanson and E. B. Working 65
Ferrari, C. G.
Report of the managing editor 294
Review of "Handbook for Bakers" by A. F. Gerhard 63
Fitz, L. A. Report of the committee on standardization of laboratory baking 252

Flour
Control of diastatic activity. R. C. Sherwood and C. H. Bailey.....107, 163
Crude protein content and relation to loaf volume. C. H. Bailey and R. C. Sherwood 393
Detection of naphthalene in. A. K. Epstein and B. R. Harris..... 60
Determination of moisture in. C. B. Morison..... 323
Effect of grade on properties of bread. C. G. Harrel..... 10
Effect of treatment with chlorine. F. L. Dunlap..... 201
Effect of sodium nitrate applied to wheat on baking quality. J. Davidson and J. H. Shollenberger 137
Factors affecting the diastatic activity of. C. E. Mangels 316
From frozen wheat harvested at various stages of maturity. W. O. Whitcomb and P. F. Sharp 301
Leavening agents in self-rising. Paul Logue and Irene T. Ranker..... 335
Missouri soft wheat in light bread. E. M. Davis and J. A. Cline..... 411
Plasticity of suspensions. J. A. Dunn 351
Plasticity of suspensions in water. P. F. Sharp..... 40
Rapid determination of gasoline color value. D. A. Coleman and A. Christie 84
Relation of baking quality to protein content. C. E. Mangels..... 150
Should it be artificially matured and decolorized? M. Javillier (trans. by Alma E. Warthen) 359
Study of the oil. C. D. Ball 19
Standard amended. U. S. Secretary of Agriculture 300
Frozen wheat
Composition when harvested at various stages of maturity. P. F. Sharp 402
Milling and baking test. W. O. Whitcomb and P. F. Sharp..... 301
Gasoline color value
Rapid determination. D. A. Coleman and A. Christie..... 84
Of several classes of wheat. D. A. Coleman and A. Christie..... 188
Gerhard, A. F. "Handbook for Bakers." Reviewed by C. G. Ferrari.... 63
Gliadin, improved method of preparation. M. J. Blish and R. M. Sandstedt 144
Harrel, C. G. Some variable factors in bread production..... 1
Harris, B. R. and A. K. Epstein. Detection of minute amounts of naphthalene in flour 60
Herman, R. S. Varying characteristics of three types of wheat grown under the influence of identical environment 244
Hermano, A. J. and O. S. Rask. Consideration of certain reactions of starches with special reference to enzyme hydrolysis 361
Hertwig-Bailey method, comparison with the official method for ashing plant tissues. C. F. Rogers 226
Hydrogen-ion concentration
Collaborative study of methods. H. E. Weaver 287
Effect of dough acidity on baking. C. G. Harrel 1
Effect on baking tests. F. L. Dunlap 205
Rapid electrometric method. H. J. Denham and G. W. Scott Blair 158
Rapid colorimetric method for crackers. R. T. Bohn and R. J. Martz.. 183
Irrigation, effect of time of, on production of crude protein in wheat. Alvin Kezer 340
Javillier, M. Should flour be artificially matured and decolorized? Summary of translation by Alma E. Warthen 359
Jones, D. Breese. A new factor for converting the percentages of nitrogen in wheat into that of protein 194
Kanred wheat, characteristics of. R. S. Herman 244
Kezer, Alvin. Effect of time of irrigation on production of crude protein in wheat 340
Kharkov wheat, characteristics of. R. S. Herman 244
Leavening agents for self-rising flour. Paul Logue and Irene T. Ranker.. 325
Lewis, J. P. and W. O. Whitcomb. The commercial protein test on wheat and some of its problems 232
Loaf volume, relation to crude protein content of flour. C. H. Bailey and R. C. Sherwood 393

Logue, Paul, and Irene T. Ranker. Leavening agents for self-rising flour 335
Malt diastase, resistance of raw wheat starch to, A. J. Hermano and O. S. Rask 374
Managing editor's report. C. G. Ferrari 284
Mangels, C. E.
Relation of protein content to baking quality of flour from hard red spring and durum wheats 150
Factors affecting the diastatic activity of wheat flour 316
Martz, R. J. and R. T. Bohn. Rapid method for the colorimetric determination of hydrogen-ion concentration of crackers 183
Methods
Comparison of the official ash method with the Hertwig and Bailey method. C. F. Rogers 226
Colorimetric determination of hydrogen-ion concentration of crackers. R. T. Bohn and R. J. Martz 183
Detecting naphthalene in flour. A. K. Epstein and B. R. Harris 60
Determination of moisture in flour. C. B. Morison 323
Graphic record of texture, volume, and contour of cakes. A. M. Child and D. I. Purdy 57
Oxygen-acetate method for the determination of ash in flour. G. L. Brendel 222
Plasticity. P. F. Sharp 40
Preparation of wheat gliadin. M. J. Blish and R. M. Sandstedt 144
Rapid determination of gasoline color value. D. A. Coleman and A. Christie 84
Rapid electrometric method for the measurement of hydrion concentration. H. A. Denham and G. W. Scott Blair 158
Rapid moisture testing oven for cereal laboratories. D. A. Coleman and H. B. Dixon 419
Report of committee on standardization of laboratory baking 252
Report of committee on methods of analysis 254
Mill products, composition of, from frozen and non-frozen wheat harvested at various stages of maturity. P. F. Sharp 402
Minutes of the 1926 convention. R. K. Durham 289
Missouri soft wheat flour in light bread. E. M. Davis and J. A. Cline 411
Mixing dough
Effect on properties of bread. C. G. Harrel 4
Mechanical modification in. C. O. Swanson and E. B. Working 65
Moisture determination
Flour. C. B. Morison 323
Study of methods. D. A. Coleman, et al 276
Testing oven. D. A. Coleman and H. B. Dixon 419
Morison, C. B. Determination of moisture in flour 323
Naphthalene, detection in flour. A. K. Epstein and B. R. Harris 60
Nitrogen, a new factor for converting the percentage of nitrogen in wheat into that of protein. D. Breese Jones 194
Oil, a study of wheat. C. D. Ball 19
Oven, rapid moisture testing. D. A. Coleman and H. B. Dixon 419
Oxygen-acetate method of ash determination in flour. G. L. Brendel 222
Panning, effect of methods on properties of bread. C. G. Harrel 2
Phosphate, effect of mono-calcium phosphate upon flour suspensions. L. Earlenbaugh 102
Plasticity
Its possibilities in cereal research. J. A. Dunn 351
of flour suspensions. P. F. Sharp 40
Protein content
Relation to baking quality of flour. C. E. Mangels 150
Of flour and relation to loaf volume. C. H. Bailey and R. C. Sherwood 393
Effect of time of irrigation on production of crude protein in wheat. Alvin Kezer 340
Factor for converting percentage of nitrogen into that of protein. D. Breese Jones 194

Protein determinations
Report of committee on methods. D. A. Coleman, et al. 254
Some of the commercial problems. W. O. Whitcomb and J. P. Lewis. 232
Proteins of flour, effect of yeast fermentation. P. F. Sharp and O. M. Schreiner 90
Punching dough, influence on properties of bread. C. G. Harrel 4
Purdy, D. I. and A. M. Child. Method for a graphic record of texture, volume, and contour of cakes 57
Quinhydrone electrode. H. J. Denham and G. W. Scott Blair 158
Ranker, Irene T. and Paul Logue. Leavening agents for self-rising flour. 335
Rask, O. S. and A. O. Hermano. Consideration of certain reactions of starches with special reference to enzyme hydrolysis 361
Registration at the twelfth convention, Denver, 1926 298
Rogers, C. F. Comparison of the official method of ashing plant tissues with the Hertwig and Bailey method 226
Sampling flour, method proposed to A. O. A. C. by H. Runkle. D. A. Coleman, et al. 280
Sampling wheat, study of. H. C. Fellows and H. B. Dixon 259
Sandstedt, R. M. and M. J. Blish. An improved method for the preparation of wheat gliadin 144
Schreiner, O. M. and P. F. Sharp. Effect of yeast fermentation on the proteins of flour 90
Secretary's report of the 1926 convention 289
Secretary-treasurer's report for 1925-26. R. K. Durham 292
Self-rising flour, leavening agents for. Paul Logue and Irene T. Ranker 335
Sharp, P. F.
Composition of wheat and mill products from frozen and non-frozen wheat harvested at various stages of maturity 402
Plasticity of simple flour-in-water suspensions 40
and O. M. Schreiner. Effect of yeast fermentation on the proteins of flour 90
and W. O. Whitcomb. Milling and baking tests of frozen and non-frozen wheat harvested at various stages of maturity 301
Sherwood R. C. and C. H. Bailey
Control of diastatic activity in wheat flour. I. Production of diastatic flour and effect of large dosages on wheat flour 107
II. Experiments with flour milled on a commercial scale 163
Relation of crude protein content of flour to loaf volume 393
Shollenberger, J. H. and J. Davidson. Effect of sodium nitrate applied at different stages of growth of wheat on the baking quality of the flour. 137
Smut, relation to protein content of wheat. W. O. Whitcomb and J. P. Lewis 236
Sodium nitrate, effect on wheat when applied at different stages of growth. J. Davidson and J. H. Shollenberger 137
Soft wheat flour in making light bread. E. M. Davis and J. A. Cline 411
Soil fertility, effect of on diastatic activity of flour. C. E. Mangels 319
Standard for flour amended. Secretary of Agriculture 300
Starch
A consideration of certain reactions with reference to enzyme hydrolysis. A. J. Hermano and O. S. Rask 361
Variation in susceptibility to diastatic attack. C. E. Mangels 320
Swanson, C. O. and E. B. Working. Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan. 65
Texture of cakes, a graphic record. A. M. Child and D. I. Purdy 57
Treasurer's report for 1925-26. R. K. Durham 292
Viscosity
Effect of calcium phosphate upon flour. L. Earlenbaugh 102
Of starches. A. J. Hermano and O. S. Rask 365
Volume of cakes, a graphic record. A. M. Child and D. I. Purdy 57
Warthen, Alma E. Translation of "Should flour be artifically matured and decolorized?" by M. Javillier 359

Wheat
A superior new variety for western agriculture. C. H. Briggs 343
A new factor for converting the percentage of nitrogen into that of protein. D. Breese Jones 194
A study of oil. C. D. Ball 19
Characteristics of three types. R. S. Herman 244
Composition of, frozen and non-frozen harvested at various stages of maturity. P. F. Sharp 402
Commercial protein test and some of its problems. W. O. Whitcomb and J. P. Lewis 232
Effect of time of irrigation of, on production of crude protein. Alvin Kezer 340
Effect of sodium nitrate on baking quality. J. Davidson and J. H. Shollenberger 137
Frozen, milling and baking tests. W. O. Whitcomb and P. F. Sharp ... 301
Gasoline color value of several wheat classes. D. A. Coleman and A. Christie 188
Missouri soft wheat flour in light bread. E. M. Davis and J. A. Cline.. 411
Rapid determination of gasoline color value. D. A. Coleman and A. Christie 84
Whitcomb, W. O. and J. P. Lewis. The commercial protein test on wheat and some of its problems 232
and P. F. Sharp. Milling and baking tests of frozen and non-frozen wheat harvested at various stages of maturity 301
Working, E. B. and C. O. Swanson. Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan.... 65
Yeast, effect of salts in bread production. C. G. Harrel 13
Yeast fermentation, effect on flour proteins. P. F. Sharp and O. M. Schreiner 90